OLFACTION

AND

TASTE

Proceedings of the First International

Symposium held at the Wenner-Gren Center

Stockholm, September 1962

EDITED BY

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FOREWORD

TODAY scientific research is recognized as being of basic importance for human welfare and culture. Every field of human activities is now the subject of research, supported by increasing financial help from governmental and private sources. Science is fundamentally international, since new facts discovered in one country are always in one way or another interesting for the rest of the world. As a consequence thereof scientists have to keep in close contact with colleagues in foreign countries by working in their institutes for shorter or longer periods, or by attending symposia and congresses.

The *Wenner-Gren Center Foundation* is intended to promote research in two ways.

1. Scientists from different countries are invited to attend symposia arranged by the Foundation. This is nowadays felt to be the most effective way of stimulating exchange of ideas between specialists in a limited research field. However, it should be noticed that the Foundation is bound to support *all* branches of scientific research without preference for any particular faculty. Natural sciences and humanities with all their ramifications are equally welcome.

2. Hundreds of research workers, most of them in the most productive period of their lives, are coming to Stockholm every year, holding fellowships from all over the world. It was felt that offering in a new form facilities for contacts between these our guests would create an extraordinary opportunity for promoting intellectual and personal international friendship and closer relations. In addition, it was considered important to give them the chance of meeting Swedish colleagues and of learning about our culture and way of living.

This was the reason why the late Dr. Axel Wenner-Gren, a well-known benefactor to science for 25 years, gave the initial capital for building the Wenner-Gren Center, and the Swedish Government generously contributed by giving a building-site to the Foundation. This is ideally situated in beautiful surroundings with practically all the important scientific institutions of Stockholm within a radius of one English mile.
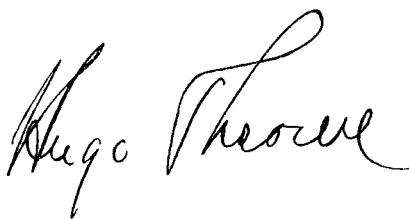
At the present time two buildings are ready and in use for one year. The 25-storey building (the " Pylon ") contains offices for many Swedish scientific authorities and organizations—The University Chancellor, State Research Councils, some institutions of Stockholm University, the Swedish Chemical Society and others. The " Helicon " in 4 storeys, over 300 yards

long, offers fully furnished flats from 1 to 6 rooms with kitchen to about
120 foreign research workers with families. At the present time people
from 30 countries are living in the Helicon.

A third building is now being planned for a restaurant, lecture rooms
and facilities for scientific and social colloquia of foreign guests and
Swedish scientists. Such activities were started in January 1962 im-
mediately after H.M. the King had graciously inaugurated the Wenner-
Gren Center. Our guests meet twice a month for scientific or cultural
lectures, and sometimes for concerts or other forms of entertainment.
The large number of children in the Helicon play their role as catalysts
of acquaintance and friendship between the families.

The two aims of the Foundation would at first sight seem to be rather
disparate. But this is not the case : in fact they are complementary.
International symposia on the highest level for research workers in a narrow
field serve specialization, which in view of the rapidly growing amount of
knowledge in all fields of science is nowadays inevitable as one of the
absolute prerequisites for progress. Specialization, however, has an
inherent danger, when driven too far, of isolating scientists to the point
where they cannot understand the language of research workers even in
closely related fields. The second aim of the Foundation, to bring
scientists in all fields and from many countries together under the same
roof is likely to counteract these undesirable effects of specialization, to
broaden their knowledge and outlook on science, to initiate and strengthen
international friendship.

With deep satisfaction we now witness our first international symposium,
on " Olfaction and Taste ", taking place in the Wenner-Gren Center
Foundation. We sincerely hope it is going to be successful in stimulating
further research in a field so closely connected with some of the mental
sources of human happiness. We also hope it will be followed by many
others in days to come.

HUGO THEORELL, N.P.
President of the Board of
Wenner-Gren Center Foundation
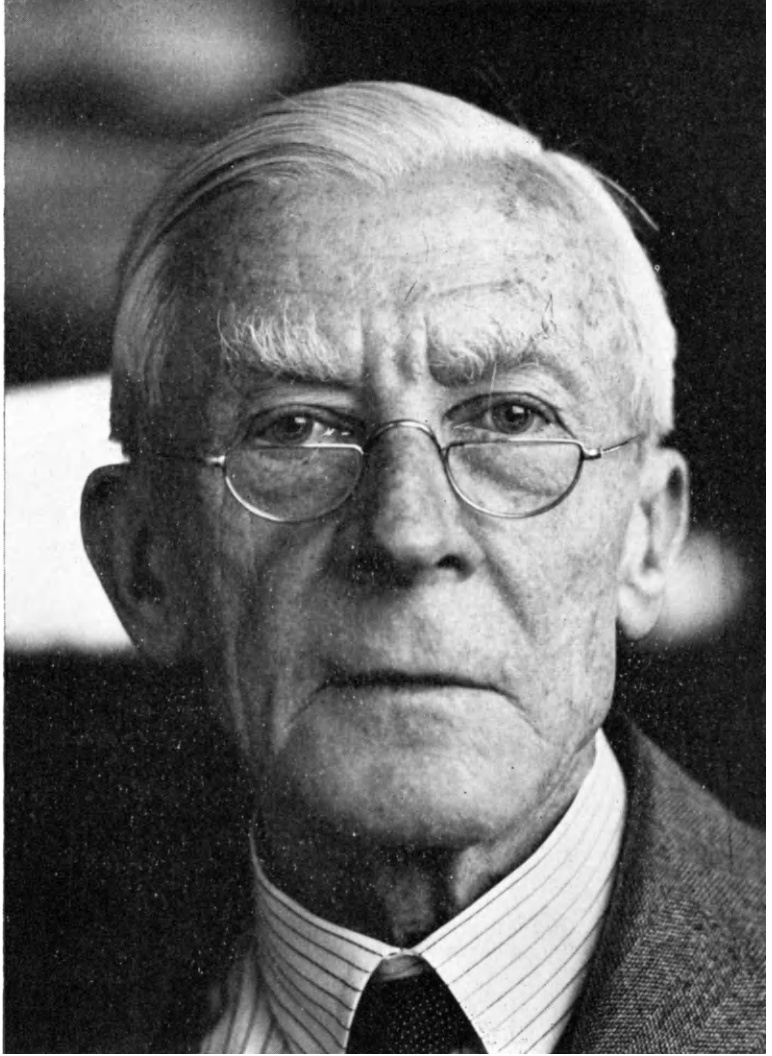
LORD ADRIAN

OPENING ADDRESS

LORD ADRIAN

I KNOW you would all like me to begin by thanking Professor Zotterman, my old friend Yngve, and the Wenner-Gren Center Foundation for inviting us to this Symposium. It is a great privilege for all of us and a particular pleasure for the very few who are old enough to have made our first visit to Stockholm for the Physiological Congress in 1926. That meeting has always been a shining example of what an international congress can be like when it has charming hosts, a beautiful city to stay in, and not too many communications to listen to.

All these conditions are satisfied at the meeting we are at now and we can expect to go away as we did in 1926 feeling that we had learnt a great deal about our subject in spite of having so much to enjoy outside it.

But our subject has reached the right stage for serious discussion and we must get down to it. It concerns fundamental problems where physiology and psychology meet : in fact we are in one of those borderlands which are the most fertile regions for the scientific advance.

The physiology of sensation began by comparing the stimulus with the sensory experience in man. It has now been enlarged to take in the intervening events, the molecular changes in the receptors and the transmission of information from them to the brain. We have to decide how the events in the environment excite the receptors and how the information is conveyed when it must show not only the intensity and time course and localization of the stimulus but the special characters which reveal its origin. The information must contain enough data to make the animal react differentially to stimuli which are of the same type and differ only in some specific quality ; and we are concerned at this meeting with a kind of information where quality is extremely important, for we are dealing with the sense organs which signal the quality of the air we breathe and that of the food and drink we propose to swallow.

With the larger sense organs in vertebrates the general quality of the stimulus would be indicated by the anatomical arrangement of the sensory system which gives each organ a special pathway to the brain. Impulses arriving by way of the optic nerve will imply a change of illumination of the retina (and will produce a visual experience) and impulses in the auditory nerve will imply vibrations of the basilar membrane.

In the skin, where the receptors are not collected into specialized groups, the character of the stimulus might be indicated by the particular type of nerve fibres which transmit the discharge. But we are still uncertain about the significance of signals in the larger and smaller nerve fibres from the skin in relation to pain and touch and so it is particularly important to learn about the arrangement of the taste receptors. These have a distribution which is half way between that of the scattered receptors in the skin and of the concentrated sheets in the eye, ear and nose. Detailed knowledge of how taste buds signal sweet or bitter will be a great help in deciding how the skin receptors signal pain or touch or temperature. And in fact it is already helping to clear up this difficult problem, thanks, of course, to the leadership and inspiration of our host, Professor Zotterman.

The eye, the ear and the nose are much more elaborate structures. They have to detect the physical and chemical changes caused by events at a distance from the body and all three give us a wide range of sensory experience. We can distinguish a vast range of sounds, a great variety of smells and innumerable visual patterns and we can do it clearly enough to give them a name and recognize their origin.

In all three sense organs the stimulus is focused on an extended sheet of receptors joined to the brain by thousands or millions of nerve fibres. With this sort of arrangement there are two possible ways in which the stimulus might give the necessary information about itself. It might produce a particular spatial pattern of excitation over the receptor surface with or without a particular temporal pattern showing the sequence and timing of the excitation in different regions : or it might excite a particular class of receptor which is specially sensitive to a particular quality.

In the ear the spatial and temporal analysis seems to be enough. The vibration frequencies in the sequence of sounds cause distinctive patterns of deformation in the basilar membrane and that pattern and its temporal fluctuation contains all the information needed to specify the sound, whether it is a pure tone, a noise or a voice or a tune that we know.

In the eye the changing pattern of light and shade on the retina gives the same sort of information and most of our visual experience is based on it : but visual stimuli vary in wave length as well as in intensity and distribution and the signalling of colour seems to involve the other kind of analysis—by receptors specially sensitive to particular wave lengths and distributed over all the colour sensitive parts of the retina.

The camera structure of the eye gives an immediate clue to the analysis by spatial and temporal patterns ; the convoluted structure of the nose is a less certain guide. The receptor surface may be several times as large as that of the retina and it lines the walls of narrow and elaborately folded passages. With a structure of this kind we should not expect to find that the different odorous molecules which are drawn through the nose at each

breath would all be deposited on the receptor sheet with the same general pattern of concentration and timing.

It is unlikely that the patterns in the nose would have as much detail and variation as those on the retina, but the great size of the receptor sheet does suggest that some of the information might be given in this way, at all events in a macrosmatic animal. And in the rabbit there are certainly distinct regional differences in the effectiveness of different odours and in the timing of the discharge they set up. In the absence of simultaneous records from multiple sites we could not expect evidence of detailed chromatographic patterns, but if we make simultaneous records from two positions in the olfactory bulb there is often enough difference to show which of four or five smells has been used to stimulate.

One can think of a variety of reasons for these spatial and temporal differences, differences in the receptors, in the nature of the surface or in the course followed by molecules of different weight and solubility in their passage through the organ. In man the organ is much less complex and we experience the same distinctive olfactory sensation whether the air is drawn in through the nose or drawn through the mouth and expelled through the nose. It is unlikely therefore that a microsmatic nose gets much information from the way the excitation is distributed, but a macrosmatic nose might get a good deal more.

But it is unlikely that these spatial and temporal patterns could be more than a minor guide in distinguishing one smell from another and so we are left with the second method, that of individual receptors sensitive to particular molecules as the colour receptors in the retina are sensitive to particular wavelengths. In the rabbit they can be demonstrated without much difficulty, for when the discharge from a single mitral cell is recorded it is usually found to be produced only by one substance or group of related substances, ethereal, esters, aromatic hydrocarbons, etc. The specificity is a fixed property of the unit, unaltered by time, anaesthesia or repeated stimulation and specific units have been found for many kinds of smell and from mitral units in several parts of the bulb.

From my own work, done some years ago, I should find it very difficult to say how many different varieties of receptor there are, but we shall hear more about it at this meeting and I think it will be clear that the part played by specific receptors in the olfactory discharge would certainly give far more information about the nature of the smell than could be gathered from the general distribution of the excitation pattern.

It seems, then, that as far as the vertebrate sense organs are concerned, our recognition of what we hear depends entirely on the general excitation pattern, the recognition of what we see depends mainly on the pattern but is aided by specific colour receptors, and our recognition of what we smell depends mainly, perhaps entirely, on specific receptors for

particular smells, though it may be aided by the characteristic pattern. This conclusion seems reasonable enough, for sound waves can only exert their mechanical effect, light waves can have different photochemical actions according to their wavelength and odorous particles will have a much greater variety of chemical effects which could be distinguished by specific receptors, though their physical properties would influence their distribution over the receptor surface.

We can only distinguish four tastes, but we can distinguish a vast number of smells : differences in the excitation pattern, if they help at all, would be unlikely to separate more than a few general categories and, although specific receptors could give much more details, the evidence seems scarcely enough to account for the whole range of olfactory discrimination. We can account for it, however, if we remember that the essential role of the central nervous system is to integrate all the incoming signals and to produce the appropriate response. Our recognition of what is happening around us is guided by all the information available whatever its source. Olfactory recognition, like visual recognition, need not depend on a single method of sensory analysis, it may depend on several methods taken together. If smells can be separated in five categories by the spatial pattern and five more by the temporal and another 50 by the specific receptors, the number of different smells that might be distinguished would be very large. We could pick them out as we pick out a book in a library catalogue by a class mark, a letter and a number.

All this is guesswork and it cannot be more than that until we know more about the reception of the taste and smell signals in the brain ; and we shall not have a complete picture of the chemical sense organs until we know how they are arranged in the invertebrate as well as in the vertebrate. Fortunately we shall hear about all these problems at our meeting. So it is high time now for me to stop making the guesses and to call on our speakers to tell us the facts.

STUDIES ON THE ULTRASTRUCTURE AND HISTOPHYSIOLOGY OF CELL MEMBRANES, NERVE FIBERS AND SYNAPTIC JUNCTIONS IN CHEMORECEPTORS*

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RECENT years have witnessed a revival of interest in sensory receptors, due in large part to the sensory physiologists. The studies of Kuffler, Fuortes, Beidler, Loewenstein and others have provided functional analyses of excitable membranes, receptive fields and feedback circuits in sensory transducers. These studies represent one of the most exciting areas of contemporary neurophysiology. Unfortunately, morphological investigations of sensory receptors and their synaptic junctions have not kept pace with the rapid advances in sensory physiology.

This is particularly true of the chemoreceptors. Contemporary textbooks and modern reviews laboriously reproduce drawings of Schwalbe, Koelliker, Kolmer and others and attempt to ascribe the properties of generator potentials, synaptic potentials and electrochemical gradients to nineteenth-century histology. To a large extent, the paucity of morphological descriptions of these regions, derives from the serious limitations of staining small unmyelinated nerve fibers with silver salts. Electron microscopy resolves most of these difficulties.

This presentation will be limited to our work on the olfactory and gustatory pathways with particular emphasis on the fine structure and histophysiology of nerve fibers and synaptic junctions. All the data have been derived from examination of rabbit tissues.

THE OLFACTORY PATHWAY

Conventional silver staining of the olfactory mucosa and examination with the light microscope reveals a great deal of information regarding the microscopic organization of olfactory epithelium. Figures 1 and 2 are

photomicrographs of olfactory mucosa stained by the Bodian silver tech-
nique. Receptor cells, sustentacular cells and basal cells can be identified.
However, the apical tips of the receptors and the fine axons comprising the
fila olfactoria cannot be resolved in any detail. Large collections of nerve
fibers (N)* are clearly evident, but individual fiber detail is not resolvable.
Close scrutiny discloses that the bipolar olfactory receptor possesses a
long dendritic process in close contiguity with adjacent supporting cells
and a terminal process or olfactory rod extends beyond the " limiting
membrane " of the epithelium. Since this portion of the neuron is the only
portion in direct contact with the external environment, a knowledge of
the structural specializations of the surface membranes might be informa-
tive to the sensory physiologist. Likewise, the distribution of nerve fiber
diameters and the organization of the fila olfactoria are important con-
siderations in any attempt to correlate structure and function.

Receptor Cells

In the electron microscope the receptor cells are clearly distinguishable
from the sustentacular cells (S) by their paucity of organized endoplasmic
reticulum (Figs. 3 and 4). Since the fine structure and organization of the
epithelium has been described in detail elsewhere (de Lorenzo, 1956, 1957
and 1960) this discussion will be limited mainly to the receptors. Figure 3
shows the apical process of a single receptor in contiguity with two adjacent
sustentacular cells (S). Membrane thickenings or desmosomes, common
to epithelial tissues, are shown by the arrows. Note however, that the
membrane thickening includes the plasma membrane of the receptor as
well. The receptor dendrite then extends as a naked process (Ro), no
longer ensheathed by supporting cells, beyond the " limiting membrane "
of the epithelium. This extension has been termed the " olfactory rod ".
The olfactory rod is cytologically interesting for several reasons. This
process contains 6 to 12 cilia in a sepal-like arrangement around the rod.
They are identical in fine structure to cilia seen in other tissues. Their
number and arrangement on the rod appear to be randomly distributed.
The plasma membrane bounding the rod membrane, consists of two dense
lines about 20 Å thick separated by a light line about 30 Å thick and is
continuous with the plasma membrane of the receptor (Figs. 3 and 4).
Quite often the membrane forms large invaginations (Fig. 4) and is con-
tinuous with a large number of vacuoles (V) which are characteristic of
cell membranes actively engaged in pinocytosis. A striking concentration

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* The following abbreviations are used throughout this article : Ca, capillary ;
G, glands ; N, nerve fibers ; Mi, microvilli ; S, sustentacular cells ; Ro, Receptor terminal
(olfactory rod) ; C, cilia ; V, pinocytosis vacuoles ; Vs, " synaptic vesicles " ; M, mito-
chondria ; Nu, nucleus ; B, basement membrane ; Sc, Schwann cells ; Es, extracellular
space ; K, mesaxons ; P, perikaryon ; A, axons ; Tb, taste buds ; Sy, synaptic junctions ;
Tr, taste receptors ; Ct, connective tissue.

of mitochondria (M) is seen in some olfactory rods, suggesting a high metabolic activity in these regions. Likewise, small vesicles of the size of " synaptic vesicles " are usually seen in the cytoplasm of the olfactory dendrite. A few filaments and occasional ribosome particles complete the cytological armamentarium of the olfactory rod. It is worth re-emphasizing that the remainder of the olfactory neuron is ensheathed by sustentacular cells and the axon subsequently by Schwann cells and *only the " olfactory rod " is bare or unensheathed.*

The apical processes of the supporting cells exhibit another type of cell membrane specialization. The plasma membrane forms numerous micro-villi (Mi) as demonstrated in Figs. 3 and 4. The cilia deriving from the olfactory rod are always enmeshed in the microvilli suggesting a possible functional relationship. Text-fig. I is a schematic representation of the olfactory epithelium incorporating our findings with the electron micro-scope. Although the olfactory receptors are considered to exhibit a high degree of physiological specificity to different odoriferous substances, our studies reveal no significant fine structural differences in the rabbit. A morphological classification for two types of receptors could, however, be made based on the presence or paucity of mitochondria in the olfactory rods (compare Figs. 3 and 4). Whether the number and orientation of the cilia constitute significant fine structural differences in receptor cells remains to be seen.
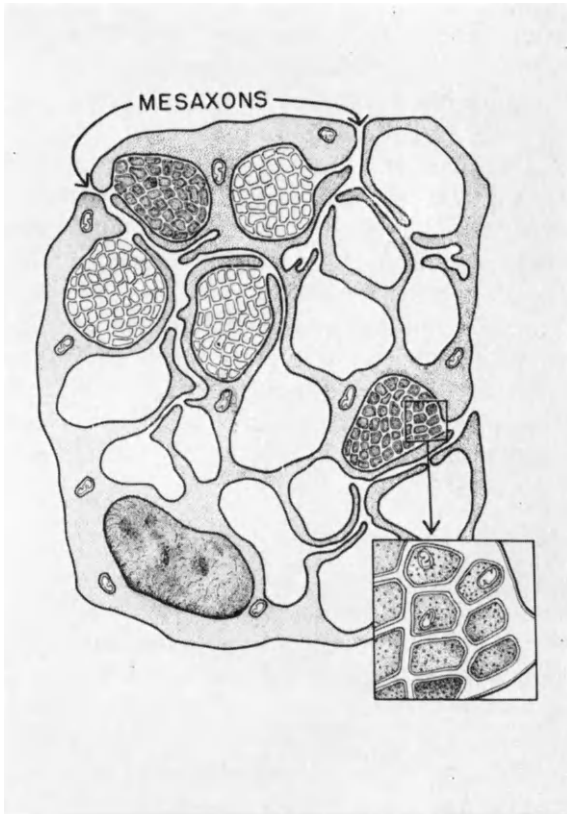
The Fila Olfactoria

The fine unmyelinated fibers which comprise the fila olfactoria of the rabbit were first described by de Lorenzo in 1956 and simultaneously by Gasser in another species. Detailed studies have subsequently appeared (de Lorenzo 1957 and 1960). The most striking features of these nerve fibers are : (1) their mean diameters which are 0.2μ and (2) their structural organization with respect to their investing Schwann cells. Figures 5 and 6 will help illustrate these features. In Fig. 5 the basal portion of the olfactory epithelium is shown. A thick basement membrane (B) separates the epithelium from the underlying connective tissue. In the upper right corner of the picture, an epithelial cell whose nucleus is labeled (Nu) is seen resting on the basement membrane (B). In the center of the picture is a group of some twenty small axons (N) collectively emerging from above and becoming embraced by a Schwann cell process (Sc). They are already organized as fascicles consisting of nerve fibers in close con-tiguity and remain so organized throughout their peripheral pathway to the olfactory bulb. Figure 6 represents a section through the fila olfactoria and here the organization of the fascicles is more evident. As has been shown in earlier publications (de Lorenzo, 1956 and 1957): (1) the mean fiber diameter is about 0.2μ. (2) The axons are packed closely together,
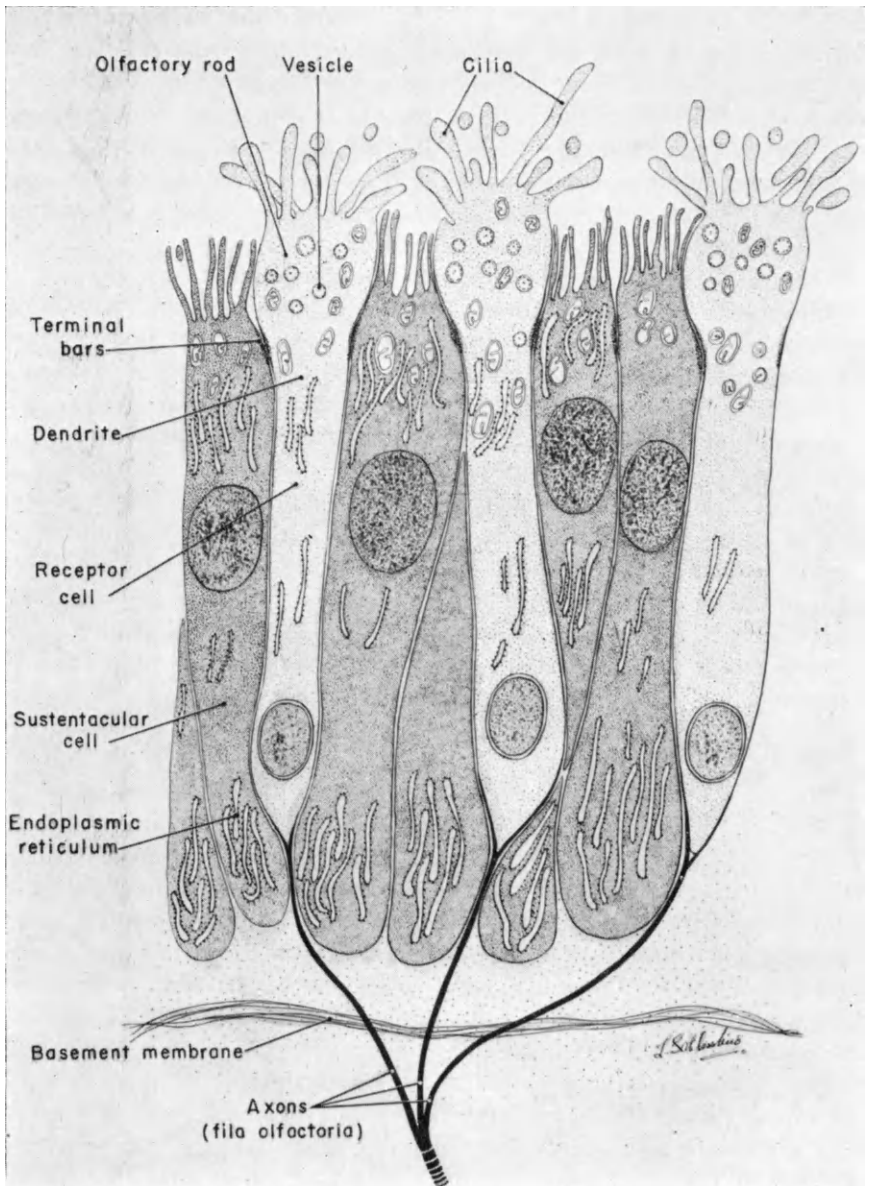
TEXT-FIG. I. Schematic representation of the olfactory mucosa showing the relationships of the various cell types based upon our observations with the electron microscope.

separated only by spaces from 100 to 150 Å wide. (3) In contrast to nerve fibers in all other parts of the nervous system thus far examined with the electron microscope, nerve fascicles rather than single fibers are enclosed

in mesaxons. Thus most of the nerve fibers in a given fascicle are not directly in contact with the extracellular environment. Figure 6 helps demonstrate this relationship. At least five nerve fascicles are evident containing numerous small axons. The regions designated by the (K) indicate two mesaxons which represent continuity with the extracellular spaces (Es). In direct contrast, all other nerve fibers thus far examined, show an individual mesaxon for each individual unmyelinated nerve fiber (compare Figs. 5 and 6 with Fig. 12). Here a single mesaxon can serve several hundred nerve fibers in a single fascicle. Since these membrane relationships may have important bearing on ion movements between the axoplasm and the extracellular spaces the structural differences have been somewhat labored. Text-figure II is a diagramatic representation of the organization of the olfactory nerve described by de Lorenzo in 1956 and 1957 and confirmed by Gasser.

TEXT-FIG. II. Schematic representation of the organization of the fila olfactoria based upon our observations with the electron microscope.

Olfactory Glomeruli

In the rabbit there are about 10^8 olfactory receptors (Allison, 1953). In all bulbs carefully examined (Holmgren, 1920 for fishes, and Cajal, 1911 for mammals) the olfactory fibers which enter the bulb do not divide before entering the glomeruli, although they do branch freely once inside. Therefore, any individual axon of a receptor cell does not terminate in more than one glomerulus and thus each glomerulus receives impulses from a distinct receptive field. Allison and Warwick (1949) estimated that in the rabbit every glomerulus receives impulses from " on an average, 26,000 olfactory receptors and passes impulses on to twenty-four mitral cells and sixty-eight tufted cells ". This arrangement offers a unique region for the study of synaptic junctions with the electron microscope, especially since silver staining of this region is most limited due to the small fibers and the structural complexity of the glomeruli. Figure 7 represents a section through an olfactory glomerulus demonstrating a large number of axon terminals. A few of these endings have been labeled (A). They are in synaptic contiguity with two mitral cells (P) and demonstrate the characteristic synaptic membrane thickening (arrows) and contain " synaptic vesicles ". Quite often axons are seen in very close contiguity ($\sim$100 Å) with each other. Although in these cases, there are no membrane specializations, " synaptic vesicles " are present in the nerve fibers suggesting a possible synaptic or ephaptic junction as reported in other systems (de Lorenzo, 1959 and 1960). The oscillations in potential reported by Adrian and others in the olfactory pathway suggest possible neural connections. The morphological evidence shows no obvious connections in receptor cells other than the desmosomes described above. In the olfactory fascicles, the close contiguity of nerve fibers ($\sim$100 Å) and the vesicles described might provide such a locus. An alternative explanation might be derived from the arrangement of the olfactory glomerulus where oscillations might be generated as dendritic potentials in the extensive glomerular network.

THE GUSTATORY PATHWAY

The serious limitations enumerated for the study of olfactory neurons are again manifest in the gustatory system. Although a detailed study of the fine structure of taste buds has been published by de Lorenzo (1958) the innervation of gustatory receptors remains obscure. The reasons for this dilemma become apparent by referring to Figs. 8 and 9. Taste buds (Tb) have been stained by the conventional silver methods. Although nerve plexuses (N) can be seen, individual axons are extremely difficult to follow even in the most successfully stained preparations (arrows Fig. 8). Identifying synaptic junctions with any degree of reliability becomes extraordinarily difficult and most times impossible. Likewise structural

details of the most interesting taste pores are not resolvable.   Again we must turn to electron microscopy.

Receptor Cells

Since our early description of taste buds in the rabbit, great progress has been made in preparatory techniques for electron microscopy.   I think it worthwhile, therefore, to briefly present some of our recent observations on taste receptors before going on to the innervation data.

The cells within the taste buds were classified as sustentacular and gustatory by the early histologists, primarily on the basis of their sizes, shapes and staining characteristics. Although most histologists find this classification useful, the electron microscopic studies suggest this is too simplified a view.   From these studies it appears that many of the cells are transitional, i.e. represent different stages of the same basic cell type. In addition various types of degenerating cells have been seen in the taste bud by electron microscopy (de Lorenzo, 1960).

Figure 10 is a relatively low magnification micrograph demonstrating the typical morphological appearance of the apical portion of a taste bud. About six receptor cells (Tr) are seen near the region of the taste pore. The apical tips of the receptors demonstrate cell surface specializations— microvilli (Mi).   The microvilli are extensions of the plasma membrane and are bounded by the typical unit membrane complex (see above). The microvilli are from 0.1μ to 0.2μ wide and two or more microns long and were first described by de Lorenzo (1958).   It is worth emphasizing that *there are no " taste hairs "* seen and therefore these " structures " seen in light microscopy must be artifacts.   The cytoplasm of the receptor is characterized by large clusters of vesicles and fibrillar material particularly in the regions near the microvilli.   Between the cells, in the region of the taste pore, are large amounts of a dense osmiphilic material that may correspond to the mucoid substance described by Ranvier.   The apical cytoplasm of the receptors contains, in addition, accumulations of mito- chondria and large numbers of dense granules (Fig. 11).   These granules are enclosed in a membrane (Fig. 11) and resemble neurosecretory and " catechol-amine granules " seen in other tissues.   The plasma membranes of the receptor cell show other kinds of specializations at their lateral surfaces.   The cells always interdigitate with each other, particularly at their apical ends (see Figs. 10 and 11). Figure 11 shows yet another cell surface specialization at the arrows—the desmosome which will be described in more detail later.   Note, however, that in this case the cell surfaces involve the sensory receptor cells alone.   Endoplasmic reticulum, RNP granules, mitochondria and Golgi apparatus complete the cytoplasmic components of the gustatory receptors.

Nerve Fibers

Myelinated nerve fibers about 1 to 6μ in diameter are seen in the connective tissue underlying each taste bud. They lose their myelin, for the most part, in the nerve plexus near the basement membrane. These unmyelinated fibers then enter the bud and establish synaptic contiguity with the receptors. The center of the bud demonstrates much more mixing of nerve fibers, deriving from many loci in the plexus, whereas the peripheral or lateral margins of the buds are more simply organized. Examination of these nerve fibers with the electron microscope quickly reveals the reason they are most difficult to resolve with conventional light microscopic techniques. Figure 12 demonstrates a region in a nerve plexus. In the upper left a gustatory receptor can be seen whose nucleus (Nu) has been identified. This cell rests upon a basement membrane (B). The spaces beneath the basement membrane are filled with connective tissue (Ct) containing collagen. A capillary (Ca) has beeen sectioned longitudinally and its lumen contains a red cell seen at the upper right. Directly beneath the capillary is a typical nerve plexus consisting of numerous unmyelinated nerve fibers (N) in close contiguity with Schwann cells and exhibiting the typical mesaxons (arrows) characteristic of most unmyelinated fibers. Note this typical arrangement in contrast to the organization of olfactory fascicles (Figs. 5 and 6). The most striking observation is the overall diameter of these fibers. There appear to be two kinds of nerve fibers— one type about 0.5μ to 1.0μ in diameter, the other below 0.5μ. Many of the smallest fibers measure only 50 millimicrons in diameter and are clearly enclosed in Schwann cell mesaxons (Fig. 12). These observations were first published by de Lorenzo in 1958. It becomes highly problematical whether these small fibers can conduct an impulse due to their minute dimensions. They may be small branches of the larger fibers.

These fibers enter the bud and assume a most unique structural relationship with the receptor cells (Fig. 13). In this figure some 30 nerve fibers, consisting of both the large and small types push into a single receptor cell, whose nucleus (Nu) is located at the right of the figure. The fibers now are embraced, as it were, by the membranes of the receptors in a manner identical with mesaxons deriving from Schwann cells. These receptor mesaxons are indicated with the arrows. Close scrutiny of this figure will reveal that all the fibers bear this relationship. Compare Fig. 13 with Fig. 12 to clarify this relationship. Some of these fibers may be terminating as evidenced by their " synaptic vesicles ". Figures 11, 14 and 15 demonstrate how the large fiber groups form synaptic junctions with receptor cells. The large fibers always seem to terminate in relationship to two or more receptor cells, i.e. they may innervate more than one receptor or may be free nerve endings, since they are between cells, near pores and more readily available to the extracellular environment. Figure 14 demonstrates

three large endings (Sy) each of which is in contiguity with the membranes of at least two receptor cells (Tr). Figure 15 is a higher magnification of a large fiber synaptic junction demonstrating some of the cytological details characteristic of this type of ending. Note that the synaptic membranes do not show any thickening characteristic of other synapses (de Lorenzo, 1959–62). However, " synaptic vesicles " are consistently seen (Figs. 11, 14 and 15). On the other hand, the small fibers end in yet another fashion. They remain in their receptor membrane mesaxons throughout their course and terminate in individual receptors rather than sharing two or more receptor cells. They do not contain large numbers of vesicles as do the large fibers, but instead contain a few mitochondria and punctate fibrils which seem to exhibit a periodicity when seen in longitudinal sections. Like the large fibers, their membranes have no
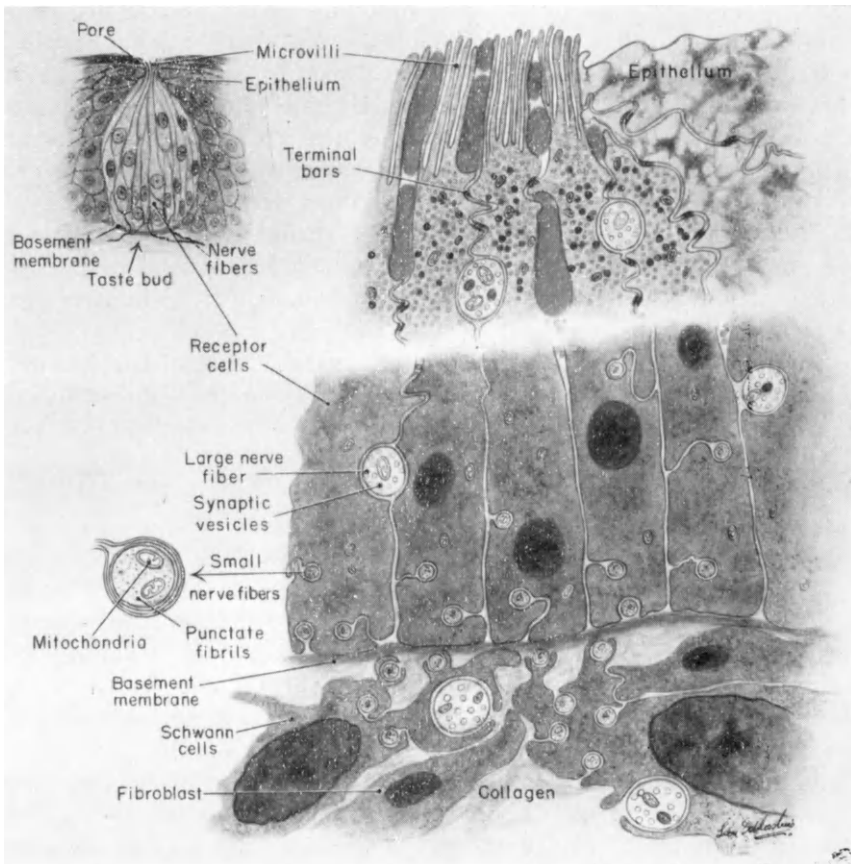
TEXT-FIG. III. Schematic representation of the ultrastructure and organization of the taste buds in the papilla foliata of the rabbit.

apparent thickening at their terminations. It has been a consistent observation that the large fibers also appear as " free endings " and thus may represent yet another type of sensory apparatus or cell type.

Several interesting questions immediately arise. Whenever two kinds of endings are seen in receptor systems one must consider the possibility of efferent endings. Unfortunately the electron microscope does not differentiate them for the neuroanatomist. Indeed it would seem most unwise to ascribe functional roles to any structural organelles without biophysical and chemical correlates. It must remain for the physiologist to demonstrate the role, if any, of the various types of endings seen by the electron microscopist. Another interesting observation is that of the so-called " synaptic vesicles " seen in the endings. If we assume, for the time being at least, that the gustatory system is chiefly sensory in nature (and the experiments of Guth (1958) support such an assumption), then the vesicles are on the post-synaptic side of the synapse. I have also reported " synaptic vesicles " in two distinct synapses that have been shown to be electrical in nature (1959, 1960b). If they contain chemical transmitters, they are most awkwardly disposed. If on the other hand, they reside in effector endings, then all is quite reasonable. Only future studies will clarify this dilemma.

Another type of receptor cell attachment is demonstrated in Fig. 16. These are the desmosomes which we also encountered in the olfactory receptor epithelium (Figs. 3 and 4). The inset in Fig. 16 demonstrates the ultrastructural organization typical of desmosomes also seen in other types of epithelium (Fawcett, 1958).

Our observations on the ultrastructural organization of the gustatory receptors, nerve fibers and their synaptic junctions are summarized in Text-fig. III.

### HISTOPHYSIOLOGY OF GUSTATORY SYNAPTIC JUNCTIONS

In our early descriptions of the rabbit taste buds, we were somewhat surprised to find that we could not clearly label each cell in the bud either sustentacular or sensory on the basis of cytological appearances. This seemed too simplified a classification, especially when we later observed many cells in the bud which demonstrated pathological changes (de Lorenzo, 1960). The work of Beidler et al. (1960) confirmed our suspicions that a cell turnover was evident. Since then, we have repeated Beidler's rat experiments in the rabbit. Table 1 represents the data derived by injecting colchicine and counting dividing cells in the taste bud germinal epithelium as described by Beidler (personal communication). Since colchicine is a most toxic substance these results must be carefully interpreted. However, they do suggest a rather rapid turnover in the foliate papillae. However, the metaphase cells were rarely found within the bud but usually around the margins (see Figs. 17 and 18). To determine whether

TABLE 1.   THE RATE OF ACCUMULATION OF MITOTIC FIGURES AFTER COLCHICINE
TASTE BUD GERMINAL EPITHELIUM

Rate 2 hr after injection		Rate 5 hr after injection	
No. of mitotic figures	Frequency of occurrence	No. of mitotic figures	Frequency of occurrence
0	11	2	4
1	57	3	15
2	30	4	26
3	4	5	31
		6	10
$x = 1.26$			
		7	11
SD 0.7			
		8	4
		$x = 4.76$	
		SD 2.18	

5 hr 4.76
2 hr 1.26
___

3.50 cells entering mitosis in 3 hr

$$\frac{3.50}{3} = 1.16 \text{ cells per hr}$$

any of the dividing cells entered the taste buds and at what rate, we injected rabbits with $1\mu c/100$ g body weight of tritiated thymidine at 1900 mc/mM specific activity.   Radioautographs were prepared for examination in the
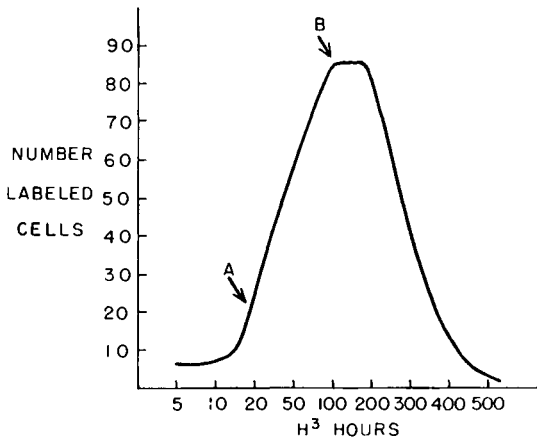
TEXT-FIG. IV.

light and electron microscope. Text-figure IV is a curve plotted from our data. At point A (about 24 hr) few cells are labeled and these are all

outside the taste bud. However, the slope of the curve from A to B represents a gradual shift of labeled cells from the margins into the taste buds. At about 100 to 200 hr a large number of labeled cells are seen within the taste buds of the foliate papillae of the rabbit. Calculating crude rates of turnover, we submit that the labeled cells migrate into the taste buds about one cell every 30 hr. Beidler's data in the fungiform papillae of the rat show a rate of migration of about one cell every 10 hr (personal communication). The rates may well vary in the anterior tongue as contrasted with the more protected posterior taste buds of the papilla foliata.

Since our results are preliminary and our experience with radioautography is limited, the rates calculated may be somewhat in error. However, on the basis of Beidler's work and our own prelimary results, we submit that the life history of the gustatory cell is quite short and that synaptic junctions are in a constant state of flux. This immediately poses many interesting problems for the neurophysiologist and neuroanatomist. Only the combined techniques of biophysics, chemistry and electron microscopy will eventually correlate structure and function in this most interesting group of chemoreceptors.

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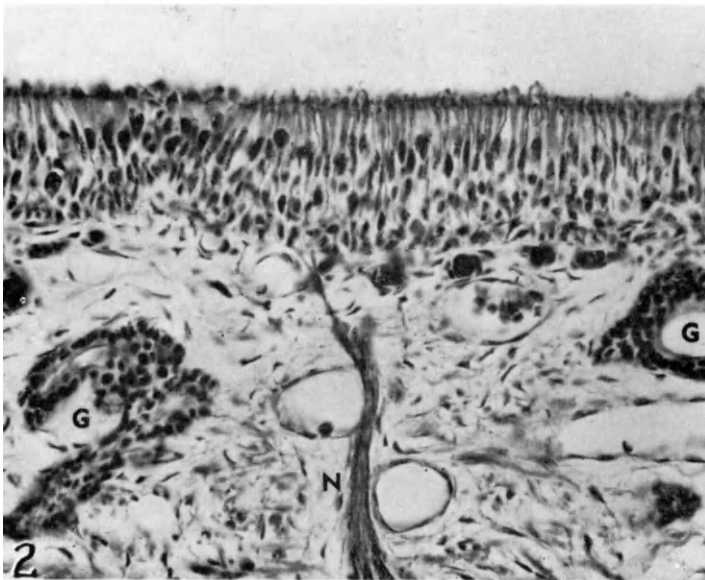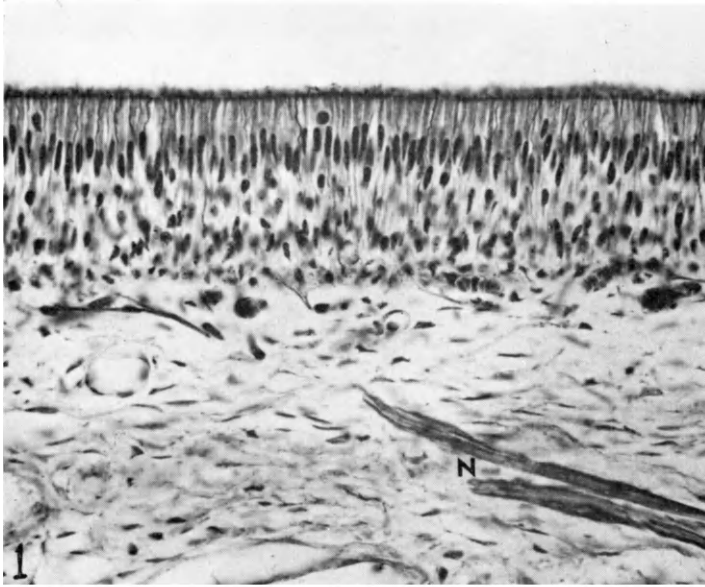
PLATE I

Figs. 1 and 2. Olfactory mucosa of the rabbit stained with the Bodian silver method showing the general architecture of the epithelium. × 600 and × 600.
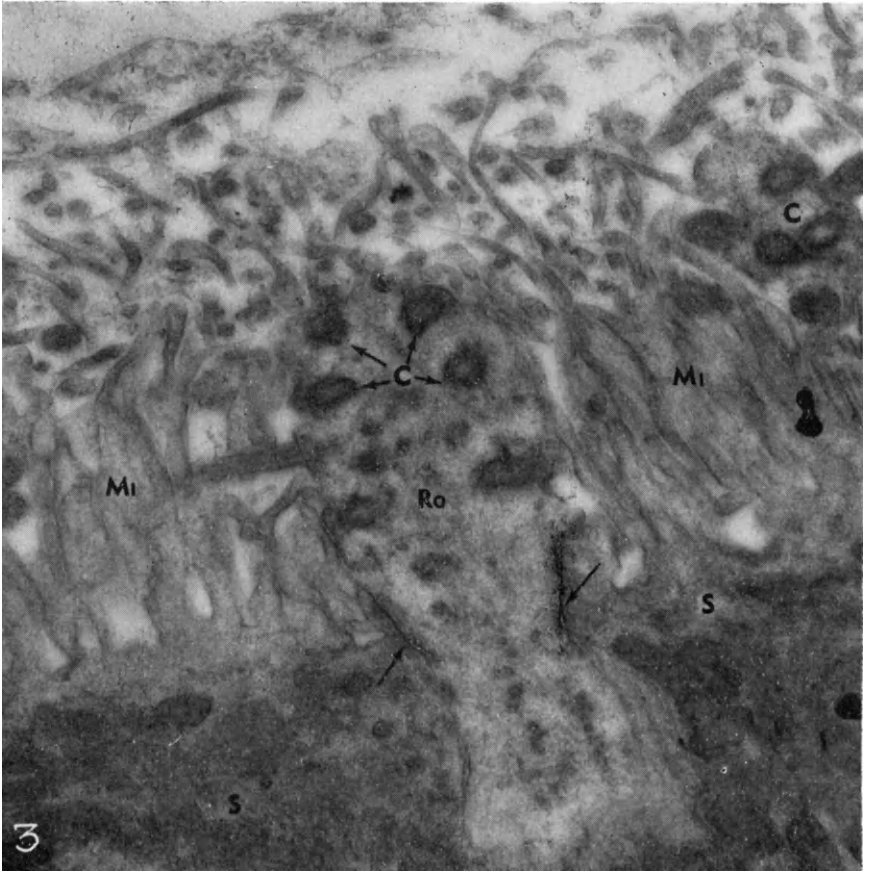
FIG. 3. Electron micrograph of the apical processes of sustentacular and olfactory receptor cells. See text for full description. Approx. × 30,000.
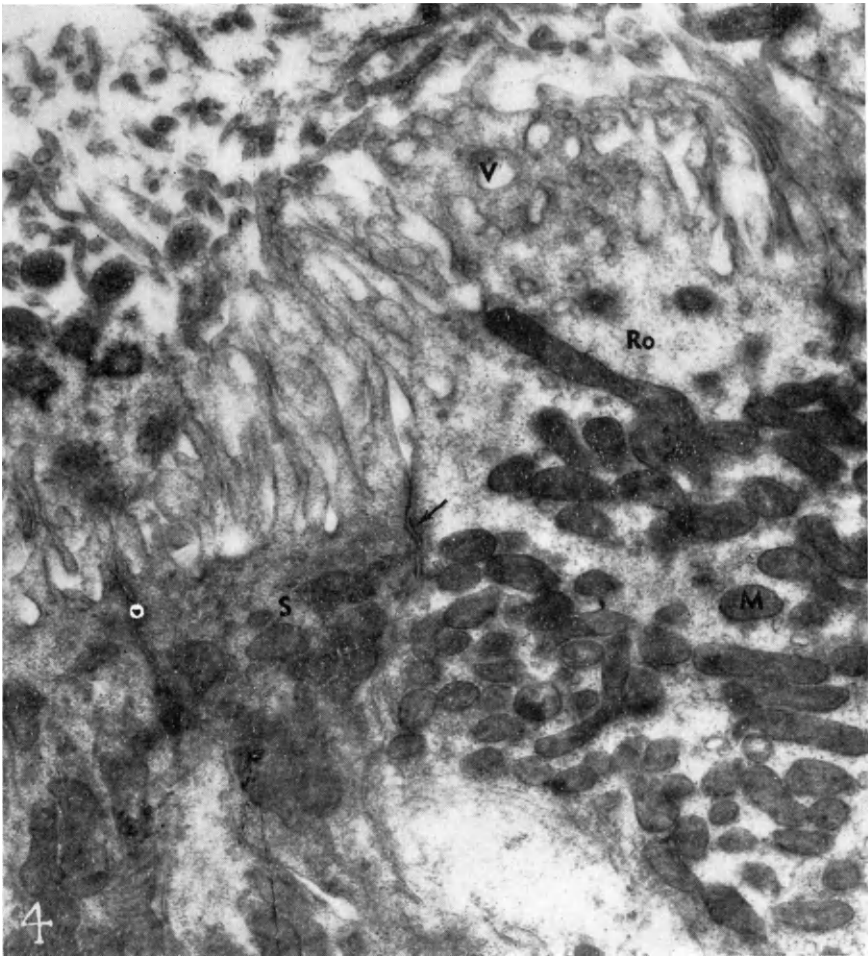
PLATE III

FIG. 4. This figure demonstrates the large concentrations of mitochondria and pinocytosis vacuoles seen in one type of olfactory receptor rod. × 36,500.
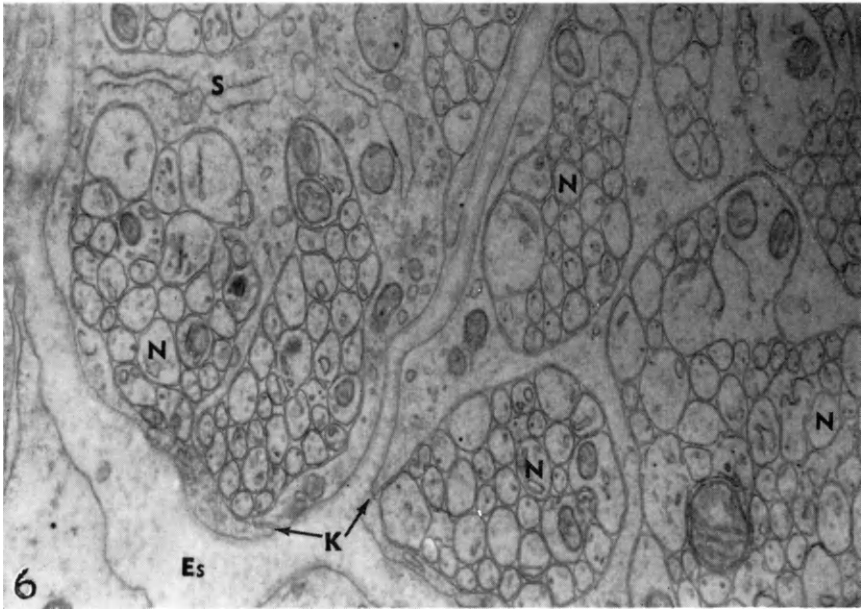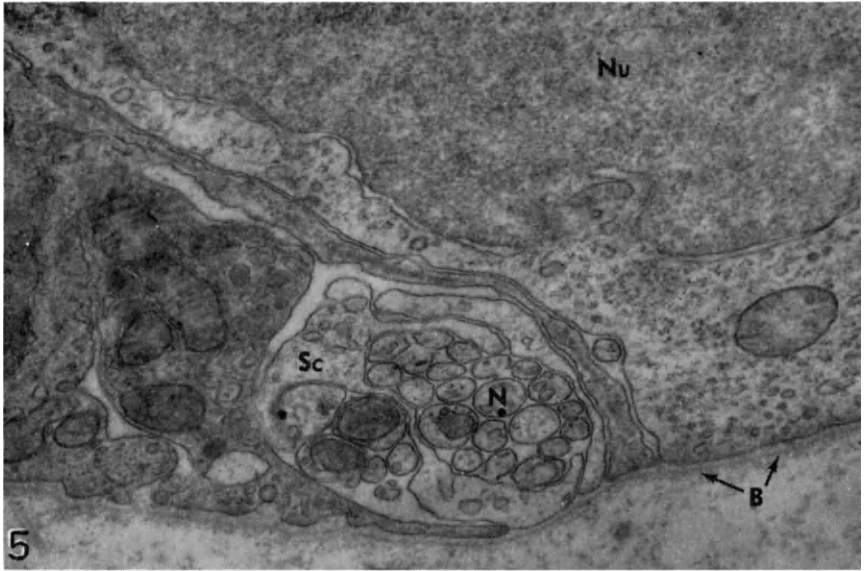
PLATE IV

FIGS. 5 and 6. These figures demonstrate the structural organization of the fila olfactoria and the sizes of the fibers comprising this nerve. Approx. × 30,000 (both).
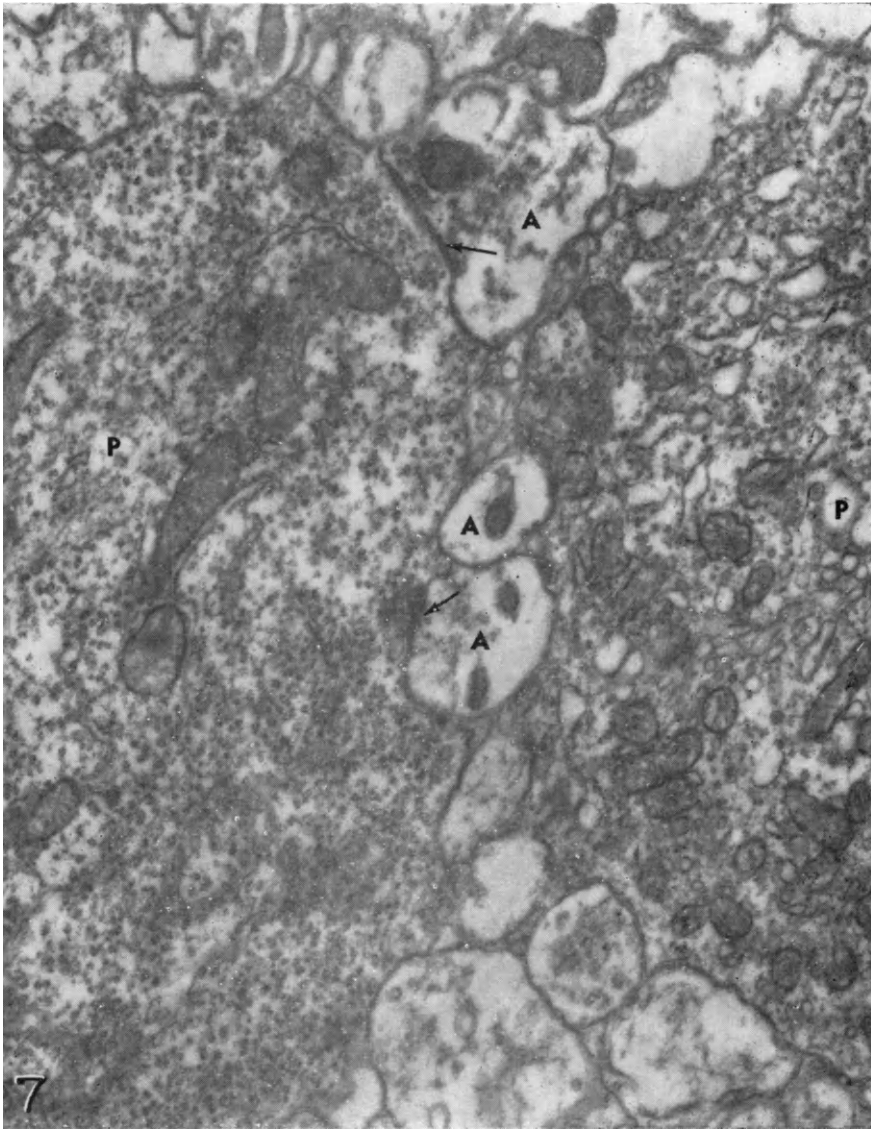
PLATE V

FIG. 7.   High magnification of axons deriving from the fila olfactoria terminating on mitral cells.   Approx. × 50,000.
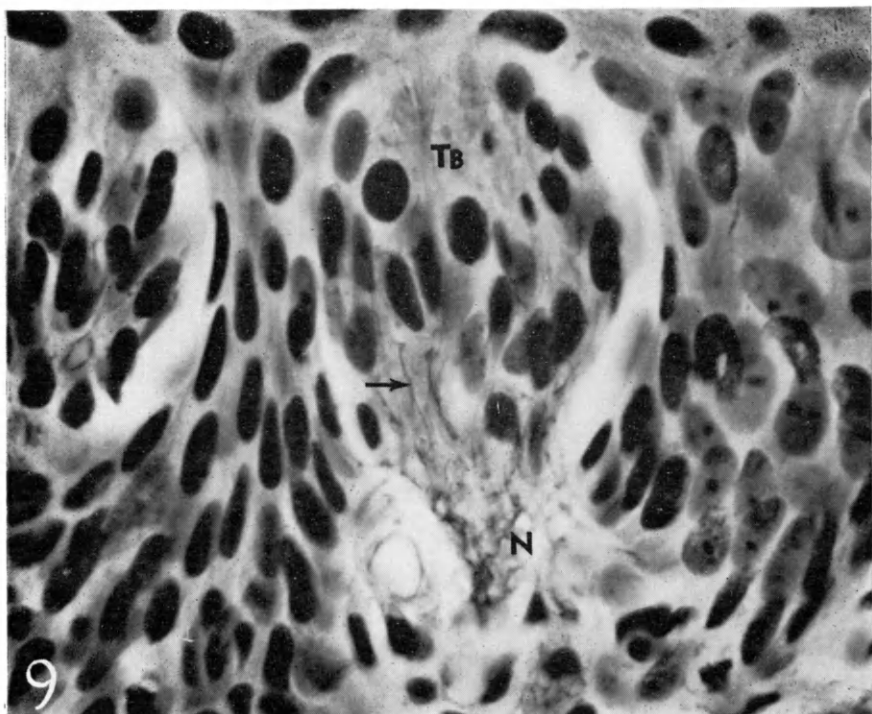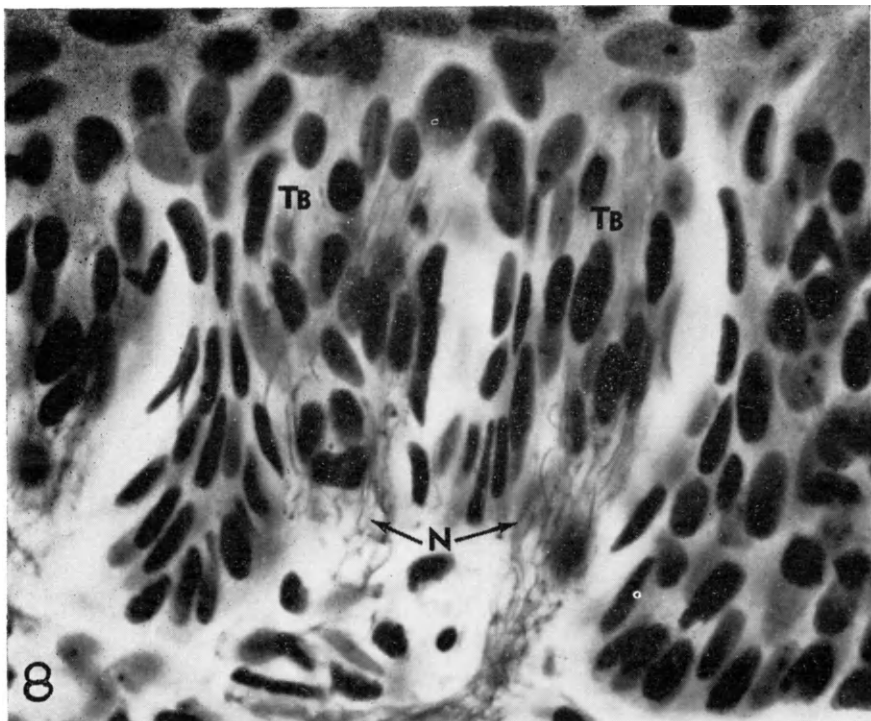
PLATE VI

FIGS. 8 and 9. Silver preparations of the taste buds in the foliate papilla of the rabbit showing nerve plexuses and small nerve fibers innervating the taste buds. Note the small fibers. × 1000 (both).

PLATE VII

FIG. 10. Low power electron micrograph showing the organization of the taste
bud in the region of a taste pore.   Approx. × 12,000.
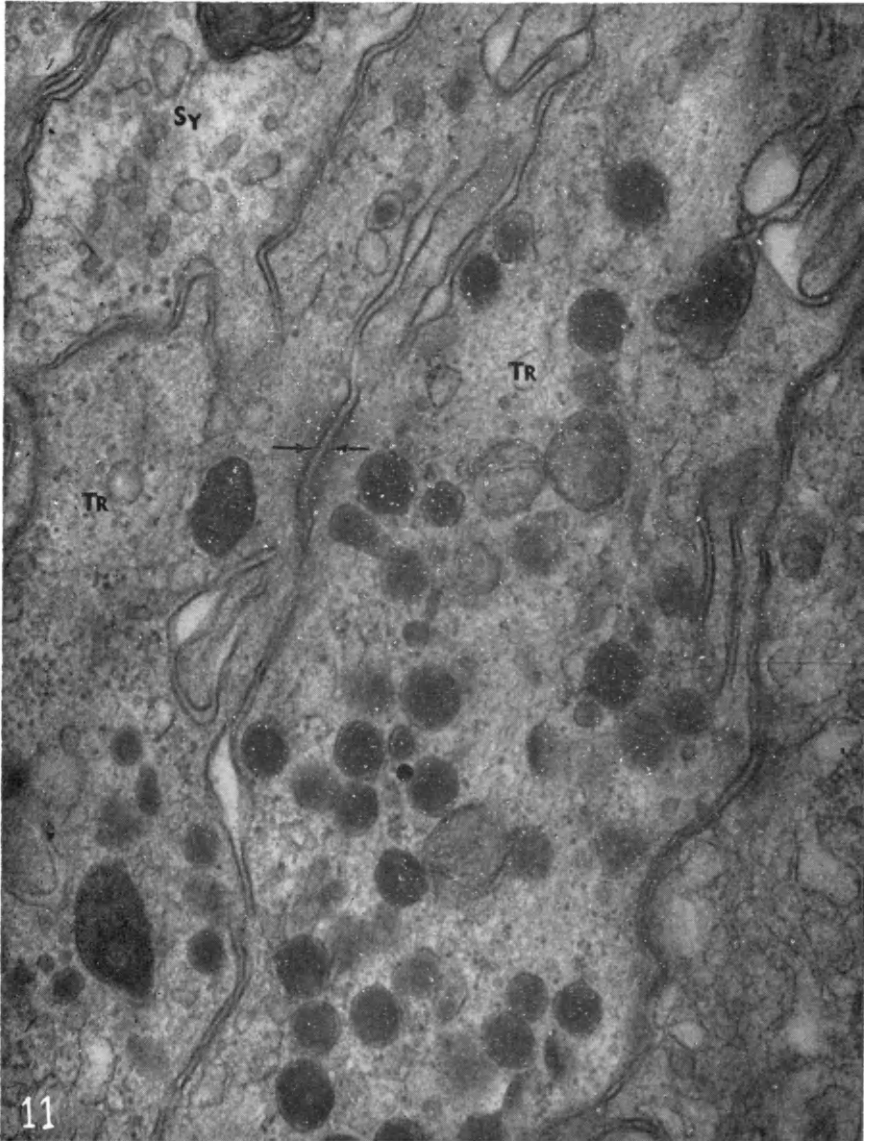
PLATE VIII

FIG. 11. High magnification of a receptor cell showing details of the cytoplasm and a synaptic junction or possibly a free nerve ending (see text). Approx. × 70,000.
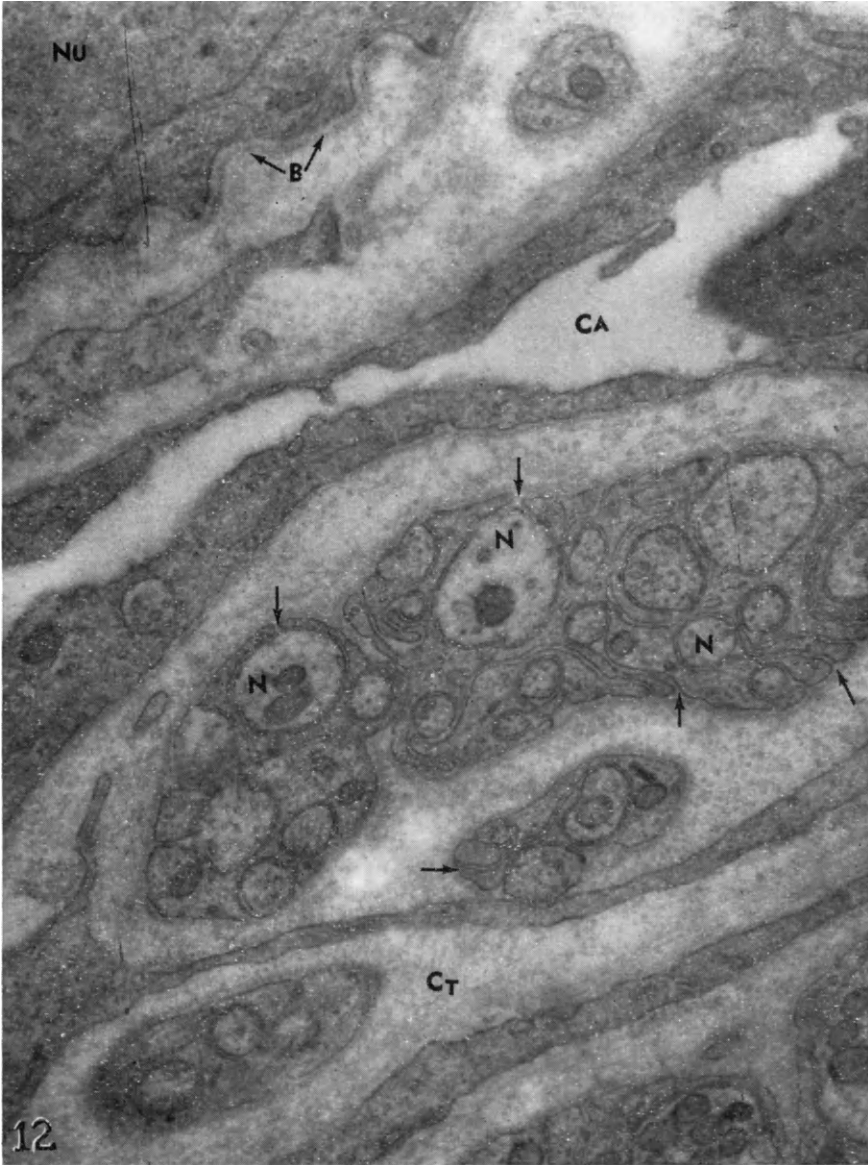
PLATE IX

FIG. 12. High magnification of nerve fibers in the plexus beneath a taste bud.
Approx. × 70,000.
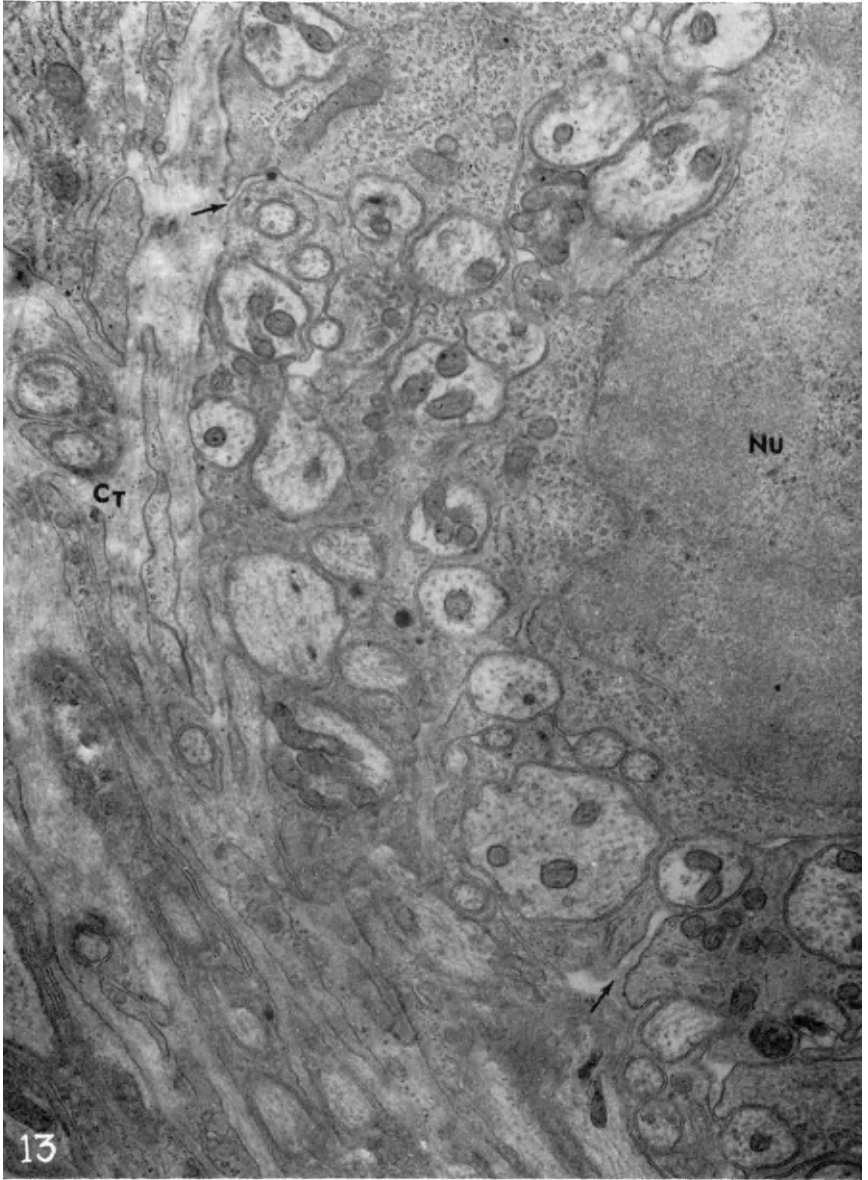
PLATE X

FIG. 13. This figure demonstrates how the nerve fibers enter the taste bud and how some of them terminate at the base. Approx. × 30,000.
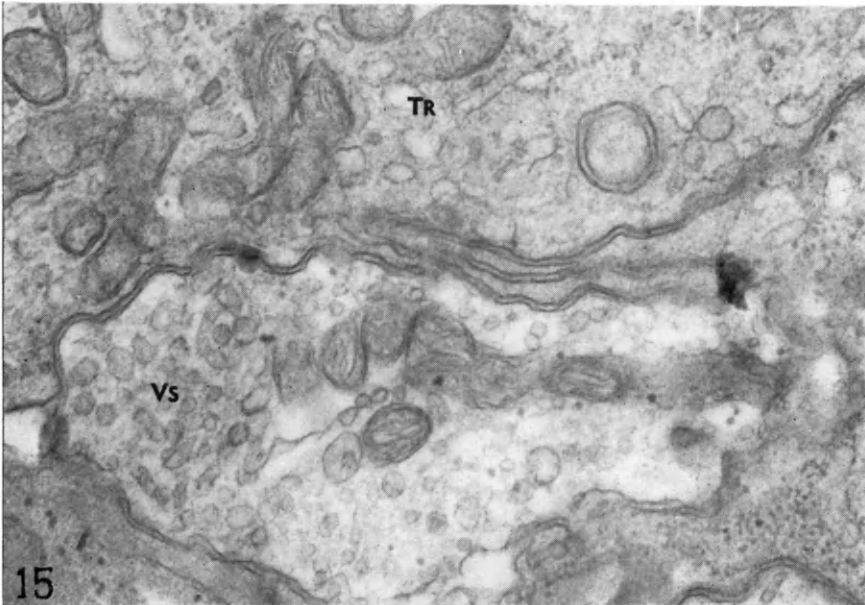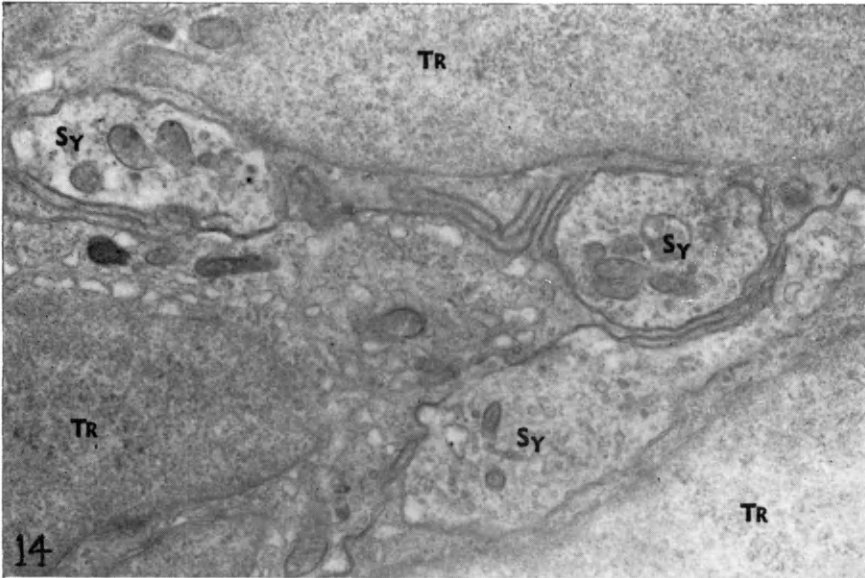
PLATE XI

FIGS. 14 and 15. These electron micrographs show the large fibers and their relation to the gustatory receptors at their terminals. Approx. × 30,000 (Fig. 14) and × 80,000 (Fig. 15).
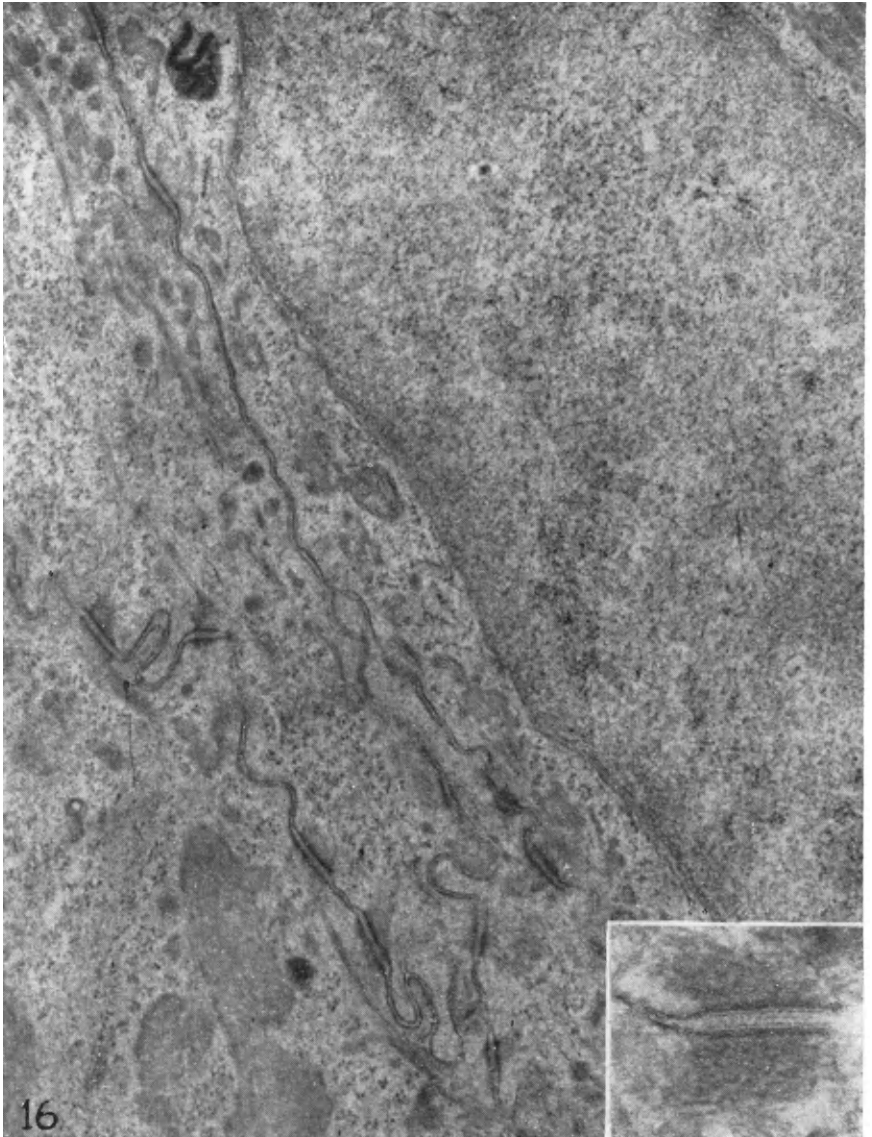
PLATE XII

FIG. 16. Membrane specializations are seen in this picture that are charac-
teristic of desmosomes seen in other tissues. Inset demonstrates ultra-
structural details of the desmosome. × 12,500 and inset × 200,000.
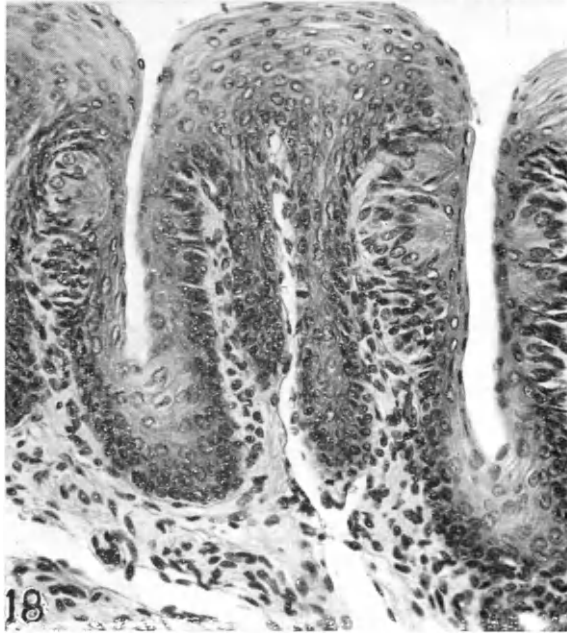
PLATE XIII

FIGS. 17 and 18.   These demonstrate the effects of colchicine inhibition in foliate papilla of the rabbit.   Note mitotic figures around but not in the taste buds proper.   × 300 and × 300.

ODOR SPECIFICITIES OF THE FROG'S OLFACTORY RECEPTORS*

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A PERSISTENT obstacle to study of the vertebrate olfactory system has been the experimental difficulty of finding out about the properties of the receptor cells. Their relatively small cell bodies, sheathed distal processes, and thin, unmyelinated axons have limited most of the experimental electrophysiological investigations to recording a large number of units at a time. Adrian's categories of some major types of odor response from the olfactory bulb (Adrian 1953) and Ottoson's studies of differences in slow potentials resulting from different stimuli (Ottoson, 1958) are the best available data. Neither allows the properties of the receptors to be described in enough detail to account for the sensitivity and selectivity of the nose. Beidler and Tucker have described a method of recording from a small bundle of axons of the olfactory nerve (Beidler and Tucker, 1955). They have not yet published the results of their experiments. Zwaardemaker's early study of cross inhibition (Zwaardemaker, 1895) provided as good a set of categories to describe psychophysical odor properties as any, but it cannot lead to a unique description of the receptor mechanism. Action potentials recorded from the second-order olfactory units located in the bulb do not show unique responses to different stimuli; this may mean that odor specificity information is coded as patterns resulting from simultaneous activity of many second-order units.

This paper describes a method of recording the action potentials of olfactory receptors by using low-impedance extracellular metal microelectrodes (Gesteland, 1961). Some of the odor-specific properties of the receptors will be described.

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METHODS

All of our experiments were done on the frog *Rana pipiens*. We used either Ottoson's preparation (Ottoson, 1956) (a decorporate frog with the olfactory mucosa exposed by removing the dorsal surface of the nasal cavity), or a curarized frog with the same exposure. Responses of cells appear to be the same with either preparation. However, the curarized preparation with intact circulation is not as sensitive to overstimulation and recovered from block caused by overstimulation more readily. Furthermore, there is a curious transition in the responses of olfactory receptors caused by the successive presentation of many odors. Most of the cells lose their specificity and become responsive to all stimuli or block and respond to none. This phenomenon does not occur as soon when the animal has intact circulation. Some of the frogs had either the first nerve or the ophthalmic branch of the fifth nerve, or both, sectioned on one side.

The animal was in a plastic box with a 1 cm × 2 cm hole for the electrodes and the stimulator in the top directly over the exposed mucosa. The frog was pinned to a cork block with a silver–silver chloride plate under his head. Deodorized moist air flowed continuously through the chamber. The box and cork were thoroughly washed and left exposed to laboratory air between experiments and had no noticeable odor.

The stimuli were small puffs of odorized air from 1 ml syringes, the plungers of which were dipped in mineral oil or ethyl alcohol solutions of reagent-grade (when available) organic chemicals. Odorized air blew directly from the syringe onto the mucosa. There was no tubing as a common path for the stimuli, as we found that it very rapidly adsorbed odors and mixed them with successive ones. The odors of the stimulating chemicals were easily recognizable, and no attempt was made to achieve such purity that we could be sure that the odor was not due to impurities. (We note the recent report that zone-refined skatole is odorless (Beynor and Saunders, 1960)). It is important to stress the significance of using very low stimulus intensities. A puff of 0.2 ml of odorized air lasting 1 sec with the syringe tip 3 cm from the mucosa will typically evoke a larger response from a unit that is sensitive to the particular odor. When stimulus strengths are so large that two successive puffs cause a decrement in the amplitude of the slow potential, the receptors will certainly be in either a generally irritable or a blocked state, and no longer odor-selective.

The EOG or Ottoson potential was monitored by a micropipette filled with 3m KCl touching the surface of the mucosa, usually at the top of the eminentia olfactoria. It indicates the arrival of the stimulus at the mucosa. The maximum sensitivity of our recording system for the slow potentials is approximately 0.2 mV for a noticeable deflection of the cathode-ray tube beam. The negative-going Ottoson potential is preceded by a small

positivity for certain stimuli. The magnitude of the slow potential depends upon the nature and the strength of the stimulus.

Action potentials were measured with a platinum-black plated, metal-filled microelectrode, coupled capacitively to a cathode follower with 30 $M\Omega$ input resistance. The indifferent electrode for both microelectrodes was the chlorided silver plate. We found that some slight variations in the procedure for preparing the metal microelectrode, which we have described previously (Gesteland *et al.*, 1959) greatly improved its ability to pick up the extracellular olfactory action potentials. We break off the tip of a glass micropipette so that it is from 2 to 5μ in diameter. Next, we extrude Cerrelow 136 alloy down the pipette to fill it to the end. If responses from the region of the axon hillock are desired, the tip is next plated with platinum black from a solution of chloroplatinic acid with a little added agar. The platinum black is first deposited slowly, then rapidly enough to cause bubbling, until a large, bushy glob is formed at the tip. The agar in the plating bath is most important. It reduces the impedance of the resulting electrode in tissue, as compared with an electrode plated from a solution without agar. If responses from the cell body or distal process are desired, it is best either to grind the tip of the electrode before plating so that it is beveled like the tip of a hypodermic needle, or to break off the tip so that the glass is jagged. Then a little alloy is dissolved out of the tip with sulfuric acid and hydrogen peroxide. Platinum black is plated to fill the hollow left by alloy dissolution. This results in a low-impedance metal electrode with a glass cutting edge to lead the way into tissue. The big, bushy ball-tip electrode was used for most of the experiments described in this paper. It will easily record from receptors singly or a few at a time, and on one occasion recorded for a few moments action potentials from a single fiber in the first nerve far from the mucosa.

A heavy micromanipulator was necessary in order to stay with units for long periods of time (2 or 3 hr sometimes).

We insert the metal electrode into the mucosa in such a way that its path is very nearly tangent to the mucosa surface. The electrode will also pick up units if it is normal to the surface, but the probability of recording is greatly diminished, no doubt because of both a reduced likelihood of contacting a unit in the optimal way and because the entering electrode is much more likely to damage the receptor terminal structures and block the sensitive area on the way in. Furthermore, the electrode irritates the mucosa in the place where it penetrates, and there is movement of the cilia and mucus as the animal tries to wash away the irritant. This may well block activity of the receptors in that area.

The metal microelectrode sees spike amplitudes ranging from the noise level of 20 μV up to 2 mV. The spikes may be monophasic, diphasic or triphasic and of either initial polarity, the local boundary conditions for

current flow set by the electrode being the determining factor. The dura-
tion of the three phases of the action potential of olfactory receptors,
extracellularly recorded, is from 3 to 5 msec if the electrode is deep, that
is, near the axon hillock or axon. Figure 1 shows three sweeps of resting
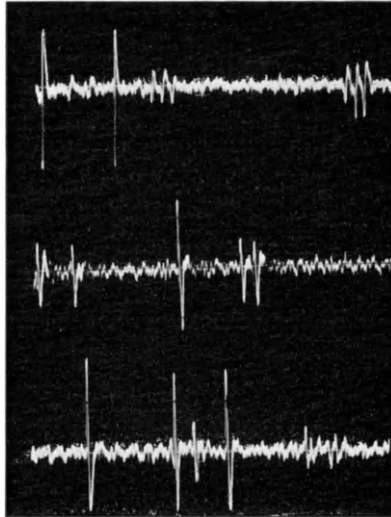
FIG. 1. Resting discharge of olfactory receptor cell axons. The lengths of the
sweeps are 0.5 sec and the largest spikes are 0.4 mV peak-to-peak amplitude.

activity of units recorded in this position. The spike duration is longer,
between 5 and 7 msec, when the electrode tip is on the mucosa side of the
basement membrane near the cell bodies, or very near the surface of the
mucosa. Action potentials recorded from fifth-nerve axons and fifth-
nerve endings in the mucosa are much shorter, approximately 1–1.5 msec,
typical of myelinated axon activity. For olfactory action potentials,
optimal amplifier frequency response is roughly from 8 c/s to 1 kc/s. The
maximum repetition rate of olfactory spikes is 20 per sec, and this is seen
only rarely when a particularly appropriate stimulus is presented to a cell.
A common rate for a responding cell is from 1 to 5 spikes per sec. The
resting rate (which may be low-level activity of the receptors caused by
room odors) is usually a few spikes in 10 sec.

Our display system operated in the following way. The vertical signal
output of a monitoring oscilloscope was used to open a gate that passed
the spike on to a second oscilloscope. The gate could be set to open only
for spikes exceeding a preset amplitude. The Ottoson potential was added
on the second oscilloscope, deflecting the base line to show the time of
arrival of the stimulus at the mucosa and its relative strength. Since two
or more units are often picked up by the microelectrode, we arranged a
sweep expansion saw tooth to be triggered by the gate so that we could

examine the shape of the spikes.   Even if two units had approximately the same amplitude, their shape was almost always characteristic, and while watching the expanded spikes, the electrode was moved slightly until different units had clearly distinguishable amplitudes.   Thus it was possible to watch simultaneously the response of two or three cells to each stimulus. Figure 2 shows the resting discharge and an expanded sweep and a normal
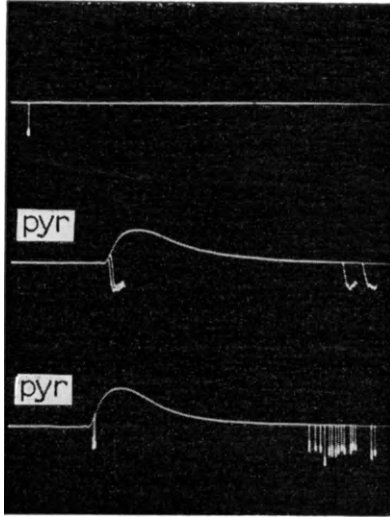
FIG. 2.   Resting discharge and two responses to pyridine.   Center sweep shows expanded spikes.   Each expansion has a 2.5 msec duration. All sweep lengths, 10 sec.

sweep of the response of two units to pyridine.   Note the different shapes of the action potentials of the two active units.   The gate also was used to provide relative brightening of the spikes compared with the base line, in order to maintain more nearly uniform photographic exposure.

ODOR RESPONSES AND SPECIFICITY

A responsive unit will generally produce a burst of spikes from 1 to 4 sec in duration.   This is usually followed by a quiet period about as long.   For a much longer time after this there is a refractory period during which the threshold of the unit is increased.   The amount of increase and the duration depend upon the strength of the preceding stimulus and its constitution.   For instance, Fig. 3 shows a unit stimulated with butyric acid twice within 1 min.   The second stimulus causes fewer action potentials than the first.   The stimulus strengths were about equal as is shown by the Ottoson potentials.   The time between the arrival of the stimulus at the mucosa and the response also depend on the particular stimulus, but not on the

strength. The usual effect of changing the strength of the stimulus is shown in Fig. 4. (We define stimulus strength as the amount of odorous
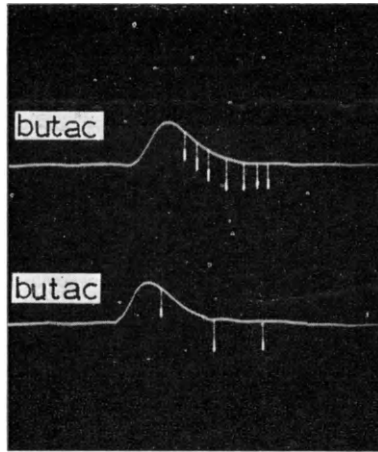
FIG. 3. Responses to two puffs of butyric acid. The lower trace was taken less than 1 min. after the upper. Sweep length, 10 sec.
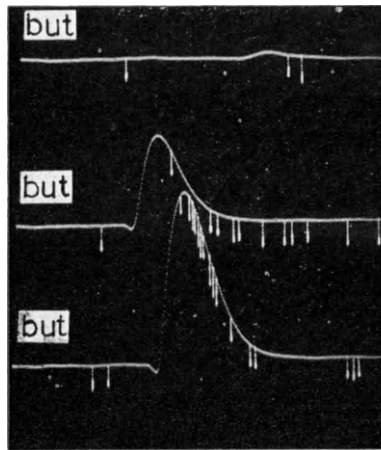
FIG. 4. Responses to increasing stimulus strengths. The top trace is the smallest; the lower, the largest puff of *n*-butanol. Sweep length, 10 sec.

substance arriving per unit time.) It shows a unit responding to three puffs of *n*-butanol. The smallest is at the top. The three records were taken far enough apart in time so that there is minimal effect of reduced sensitivity because of the preceding stimulus. The pattern of the response is strikingly similar in all three cases, even though the number of spikes increases with stimulus strength. There is always a threshold effect, and for a unit that has not had its threshold raised by multiple preceding

stimuli, the threshold is below the level at which an Ottoson potential can be distinguished. Overstimulation causes extended high thresholds. If the unit is overstimulated by several different stimuli, it will often discharge at a very high rate for many seconds, and then go into a state with prolonged high threshold. It is not a dead receptor, however, and will respond with a few spikes to an appropriate stimulus. After a long period of stimulation, even when the stimuli strengths have been kept low enough to avoid these effects, many of the units that we record are generally irritable, responding strongly to all successive stimuli. The mucosa does not return to its initial or normal state after this effect has set in. An interesting phenomenon is seen if a puff of cigarette smoke is blown at the exposed mucosa with the decorporate preparation. There is a big increase in background activity (activity of cells too far from the electrode tip to be distinguished from noise) which suddenly becomes an oscillation of 5–10 c/s. This oscillation lasts for a few seconds and is apparently phasic activity of many receptors. The receptors do not show much activity or selectivity after such an oscillation has occurred. However, it can be obtained repeatedly, and the frequency of the oscillations changes somewhat with composition of the smoke. We do not get the oscillations when circulation is intact. The dc potential of the mucosa becomes very erratic sometime after single units show the effects of massive stimulation.

When we section the olfactory nerve and let degeneration take place for a week or more, the effects on the Ottoson potentials and single-unit responses are very apparent. The Ottoson potential is reduced to approximately 50 per cent of the amplitude recorded from the mucosa with an intact nerve, and the frequency with which one can find a unit with an electrode is markedly reduced. With careful exploring, spikes can be found and they are odor-specific in their responses. This agrees with Le Gros Clark's histological studies on degeneration following first-nerve section in the rabbit where he found at least half of the olfactory receptor cells to have degenerated (Le Gros Clark, 1957). Section of the fifth nerve, on the other hand, has no obvious effect on the Ottoson potential, amplitude, and the number of active single units or their response properties. On a few occasions with a preparation with intact fifth nerve we have encountered a single unit in the mucosa with an action potential that is short compared with olfactory units, approximately 1–1.5 msec. Figure 5 shows such a unit. The large spike has a short duration, and the small one is more than twice as long. The top trace shows the resting rate, the middle trace shows the large unit responding to butyric acid, and the bottom trace, a weak response of the small unit to musk xylene. The large unit showed some response to camphor and mercaptoacetic acid, but to nothing else, even if very strong puffs were used. The small unit responded to a larger group of odors. It seems most likely that the large unit is a fifth-nerve ending.

4

A few times, we have recorded from a metal microelectrode inserted in the ophthalmic branch of the fifth nerve. Here also the spikes are of short duration, and the units responded to the onset of heat with a decrease in rate, and to turnoff of heat with an increase in rate compared with the
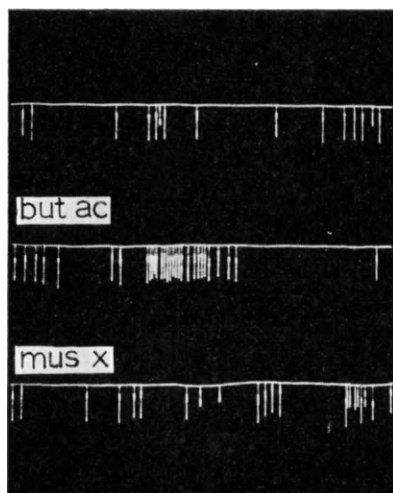
FIG. 5. Resting discharge and responses to butyric acid and musk xylene. The large spikes have a short duration. The small spikes have the usual longer duration that is typical of olfactory receptors. Slow potentials are hardly visible because of low stimulus strength. Sweep lengths, 10 sec.

resting rate. The units responded to touch with a rate increase. We did not get responses to irritating chemicals but we have not tried often to find such responses. Olfactory units recorded with the electrode as in the usual preparation do not respond to small variations in temperature and probably not to touch.

The olfactory receptors are all odor-selective, that is, each one responds to certain of the odors to which it is exposed and does not respond to others. Most show a strong response to at least one of the twenty-five odors that we have used and a weaker response to many more of them. Figures 6-14 are examples of odor-specific responses of some of the cells that we recorded.

From these records and many more, we can suggest that there are some patterns that are present in the responses of different cells, and we can begin a list of the different groups of receptors. Our list is characterized by extensive overlap, as if chemical names were not a good way to characterize these types. However, odor properties do not seem to be any better. One group responds vigorously to limonene, camphor, pinene, and somewhat less to carbon disulfide. A second responds to coumarin and musk. Group three responds to butyric acid, valeric acid, mercaptoacetic acid,
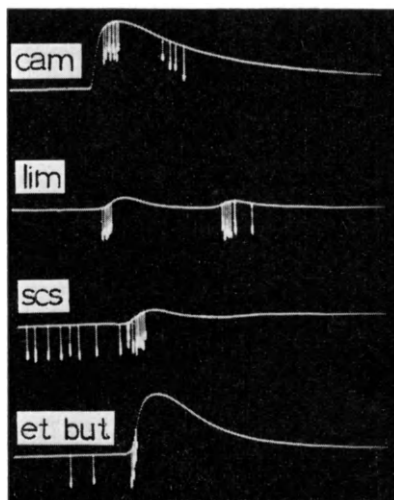
FIG. 6. A unit that responds strongly to camphor, two puffs of limonene, carbon disulfide, and slightly to ethyl butyrate. Sweep lengths, 10 sec. Note early response to carbon disulfide because of odor on the outside of the syringe as it was brought into position. A lower amplitude spike also responds to camphor in the top trace.
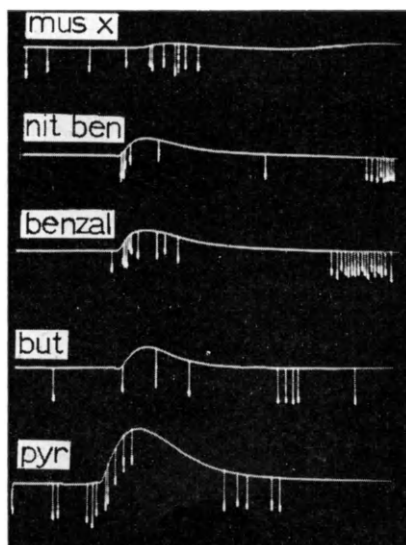
FIG. 7. More responses from the same recording position as Fig. 6. Top trace shows a weak response of the two units to musk xylene. Below it, the one unit shows long-delayed responses to nitrobenzene and benzaldehyde. The two bottom traces show a larger unit, which was not responsive to the preceding stimuli, slightly responsive to *n*-butanol, and more so to pyridine. Sweep lengths, 10 sec.
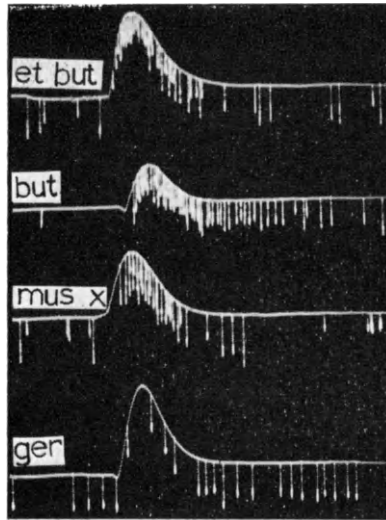
FIG. 8. Two units show vigorous responses to ethyl butyrate and *n*-butanol. Vigorous response of a different larger unit and one of the smaller units to musk xylene. Weak response of the larger unit to geraniol. Sweep lengths, 10 sec.
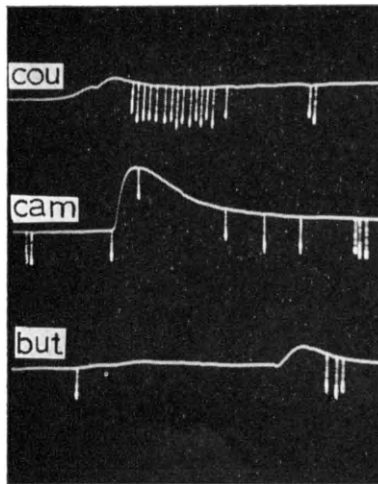
FIG. 9. Two units of different amplitude are active in these three traces. One responds to none of the three stimuli. The other responds vigorously to coumarin, and weakly or not at all to camphor and *n*-butanol. There is a continuous difficulty of separating a weak response from the usual highly erratic resting discharge rate. Sweep lengths, 10 sec.
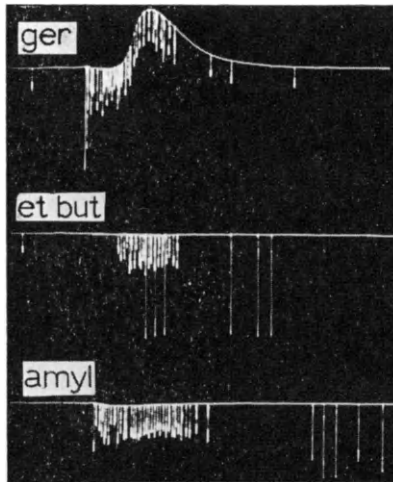
FIG. 10. Several units with clearly different amplitudes can be seen in all the traces shown here and in Fig. 11. A small unit responds vigorously to geraniol, ethyl butyrate, and amyl alcohol. A smaller unit is active as well for geraniol. A large unit responds slightly to ethyl butyrate. This unit also responds to amyl alcohol slightly, as does another slightly smaller unit. All sweep lengths, 10 sec. Except for geraniol the stimuli are too small to discriminate the slow potential.

FIG. 11. From the same position as the traces shown in Fig. 10, the two large units respond to benzaldehyde, benzonitrite, and musk xylene. There is little or no response of the small units to these stimuli. Same sweep length as in Fig. 10.
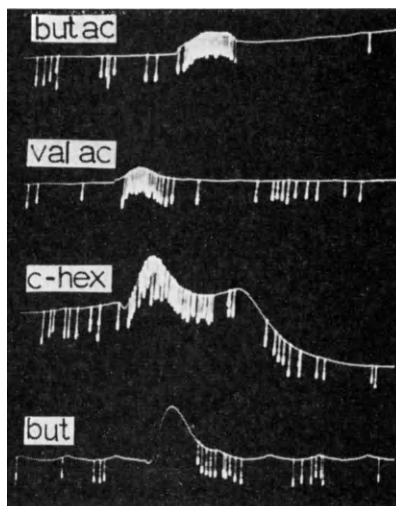
FIG. 12. A unit is shown in these four traces which responds strongly to butyric acid, valeric acid, and cyclo-hexanol. There is a weak response to *n*-butanol. Base-line drift is caused by movement of the micropipette electrode. Sweep lengths, 10 sec.
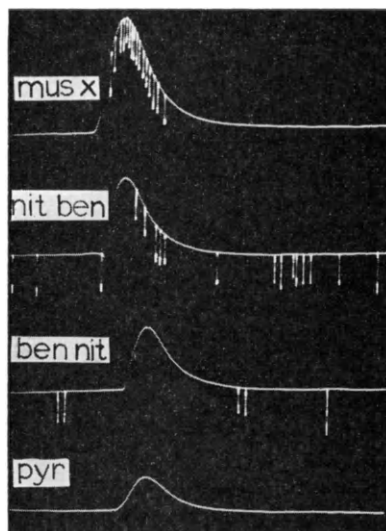
FIG. 13. A unit that responds to musk xylene, slightly to nitrobenzene, less so to benzonitrite, and not at all to pyridine. Sweep lengths, 10 sec.

and cyclo-hexanol. Group four responds to benzaldehyde, nitrobenzene, benzonitrite, musk, and amyl alcohol. Group five responds to pyridine, musk, cinnamaldehyde, and *n*-butanol. Musk is the strongest stimulus for a sixth type that does not show much response to benzaldehyde or nitrobenzene. A seventh type responds to pyridine more strongly than to most of our other stimuli. The eighth group, which is very common, responds to butanol, ethyl butyrate, amyl alcohol, and geraniol. There seem to be other types but we have not seen them often enough to be able to characterize them at all. Furthermore, it is possible and likely that these types may be condensed into fewer groups or expanded into more. If we have not used stimuli that are especially effective and, instead, are seeing responses to some of the large number of odors that weakly affect a type, it would account for much of our uncertainty and for the fact that no two units seem to be completely alike.
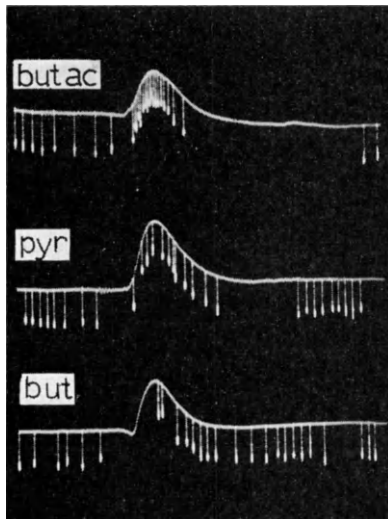
Fig. 14. A unit that shows a strong response to butyric acid, and weak response to pyridine and *n*-butanol. It could also be interpreted as showing inhibition for *n*-butanol. Sweep lengths, 10 sec.

To give some indication of the complexity that we face, we have compiled the table shown in Fig. 15. It is a list of all of the stimuli that we have used in many experiments. The second column lists the number of cells that we attempted to stimulate with each odor. The third column is the number of cells that showed a repeatable response measured as a transient increase in discharge rate. Most units respond to many things. Each cell shows such individuality in its weaker responses that, in spite of a rather large number of attempts, we have not been able to discover a unique set

of odors that accurately describes the selectivity of a limited number of receptor types. However, judging only on the basis of strong responses, it appears to us that there is a limited number of types of receptors.

Stimulus	Cells sampled	Cells responding
n-Amyl Alcohol	25	14
Musk Xylene	38	20
Benzaldehyde	36	19
Benzyl Acetate	12	6
Geraniol	18	9
Benzonitrite	22	11
Pyridine	32	15
Indole	19	9
Camphor	32	14
Methyl Salicylate	18	8
Butyric Acid	30	13
Linalool	14	6
Pinene	7	3
n-Butanol	47	19
c-Hexanol	20	8
Nitrobenzene	42	16
Triethylamine	14	5
Ethyl Butyrate	31	10
Mercaptoacetic Acid	19	6
Valeric Acid	13	4
Limonene	17	5
Coumarin	22	6
Carbon Disulfide	28	7
Cinnamaldehyde	25	6
Methyl Anthranilate	6	1
Salicylaldehyde	21	3

FIG. 15. The stimuli used in various experiments, the number of cells on which each was tried, and the number of those which responded with an increase in discharge rate are tabulated here. Weak and strong responses are lumped together.

DISCUSSION

The chemical selectivity of the olfactory receptors in the frog's mucosa which we see is a curious and unsatisfying kind of selectivity. It is analogous to a collection of poorly constructed optical filters. We could describe the optical filters as follows. No two are quite alike. The response spectrum changes with repeated use and environmental conditions. The transmission band has many notches in it and the sides of the transmission band fall off slowly and irregularly. Yet, the yellow ones are clearly a group apart from the red ones. We do not suggest that nature

has in fact given the frog such an inferior set of analyzers for odor. However, our measurements are apparently made in such a way that we cannot discern the unique properties for which we are searching. The categories that we might construct do not fall into order on the basis of simple chemical properties, or on any psychological odor groupings. There are no data on cross inhibition either at the behavioral or physiological levels for the frog, and data from other vertebrates do not help in achieving this order either. In our earlier experiments in which we used only a few odors, one from each of Zwaardemaker's major groups, it appeared that many units were uniquely sensitive to only one odor of our set. However, when we expanded the collection of stimuli, the exceptions were much more common than were the ones that responded according to our supposition.

If we assume (as appears reasonable from our data) that the resting discharge rate for the receptors is either small or zero, and therefore that inhibition is not a major part of the code for describing an odor, we can consider several different possible types of receptor mechanisms and see if any are preferred by reason of being consistent with our measurements.

1. All of the receptors are identical. In this case the number of active cells would indicate the intensity of the stimulus and the pattern of the discharge or the topographical position of the receptor on the mucosa or both would indicate the quality of the odor. Under these assumptions, we should see very similar responses to the same odor from one cell to the next, at least when the electrode stays within a restricted area of the mucosa. In fact, of course, the receptors that we record do not behave in this way at all. Differential selectivity is most obvious when the electrode is recording simultaneously from different units with clearly discriminative amplitudes.

2. There are several different species of receptor cells, each species of which has particular selectivity properties. If the receptors are like this, and if our recording method does not seriously disturb the properties of the units, we ought to see, at least occasionally, two cells that respond in the same way to our entire collection of stimulants. In fact, there is a difference in the response of any two cells with respect to some odors. It is possible that we have seen some identical cells but have disturbed their identity by our manipulations. More sophisticated experimentation might prove this to be the correct description.

3. There are a great many different receptor types, possibly one for each odor or combination of odors. This seems unlikely, as we see a strong tendency for the receptors to form at least vague odor groups.

4. There are different receptor site types that are distributed over each cell. One cell can differ from the next in having different ratios of the receptor sites. In this case the variability that we see is most reasonable, in

that whether a receptor responds or not depends on the relative occupa-
tion of the different sites, or on occupation of a minimum number of sites
of more than one type. Some sites could be inhibitory, but it is not neces-
sary to postulate this in order to have a useful code without great numbers
of different cell species.

At present, the fourth receptor model seems the most compatible with
our data, but we are in no position to settle the question yet.

SUMMARY

1. A new recording technique that allows the action potentials of single
primary olfactory receptors in the frog to be recorded extracellularly has
been described.

2. The general response patterns of the olfactory receptors have been
described and correlated with the observations on the slow potentials of
the mucosa.

3. Some of the odor-specific properties of the receptors have been
described.

4. Possible receptor mechanisms have been discussed in the light of the
new data on odor-specific responses.

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GENERATION AND TRANSMISSION OF SIGNALS IN THE OLFACTORY SYSTEM

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THE olfactory receptor cell is a bipolar neuron with a peripheral extension, from which a number of hairlike filaments protrude into the mucus covering the epithelium. The afferent nerve fibre emerges from the proximal pole of the cell and runs together with fibres from adjacent cells in small fascicles to the brain. This small neuron and its processes exhibit some rather remarkable functional features. It is able to detect minute amounts of certain substances and to transduce the stimulus into a coded message that provides the brain with information about the nature and strength of the stimulating agent. How the cell carries out this function has for long been a challenging problem to physiologists and biochemists. It is the aim of the present paper to discuss some of the problems concerning the transformation of the events in the receptor membrane into the electrical signals carried to the brain.

One of the basic problems in the study of the function of the olfactory sense organ concerns the question of which part of the primary neuron serves as the sensory element. Various parts of the cell, such as the olfactory rod, the vesicle or the hairs, have been suggested to represent the chemosensitive portion of the cell. The experimental evidence supports the view that the primary reaction between the stimulating agent and the neuron takes place in the membrane of the hairs. The morphological features of these structures seem to vary greatly from one species to another. In the frog the hairs have a length varying from 20 to 200μ and form a feltwork of densely interwoven filaments close to the surface of the mucus layer. This feltwork may be regarded as the sensory membrane and chemoelectrical transducer of the olfactory apparatus.

In spite of all the efforts to evaluate the mechanisms underlying the action of the odorous particles on the receptors we still don't know what is the essential stimulus. A number of theories have been advanced which assume that the olfactory membrane is excited by some sort of radiation emitted from or absorbed by the particles. Experimental evidence speaks strongly against these theories. It has, for instance, been demonstrated (Ottoson, 1956) that the olfactory receptors cannot be excited when they

are separated from the odorous particles by a thin membrane. We may therefore safely assume that in order to excite, the odorous particles must be brought into contact with the receptors.

The events that take place in the sensory membrane after the particles have reached the receptors have been the subject of a great deal of speculation. It seems most likely that the first step in the excitatory process involves the adsorption of the molecules on the membrane. It has been suggested by Beets (1962) that this stage also includes an orientation of the molecules so that they fit into certain sites of the membrane. It is generally recognized that the steric configuration of the molecule is of essential
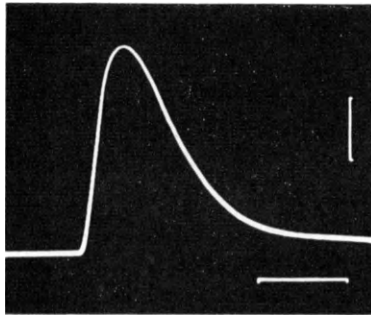
FIG. 1. The electro-olfactogram. Response obtained from the frog's nasal mucosa to stimulation with butanol vapour. Vertical line 1 mV. Time bar 2 sec. (From Ottoson, 1956.)

importance. At the present state of knowledge no definite conclusion can be drawn as to the interaction and relative significance of the various factors which may influence the action of the substance on the end organs. In discussing the processes that may precede excitation, the possibility has also to be taken into consideration that the action of the stimulating agent may be mediated by the release of some intermediary substance. It has been suggested by Nachmansohn (1959) that ACh might participate in the normal process of excitation of sensory endings. Experimental tests on the effect of ACh on various types of sensory end organs have shown that ACh initiates a discharge in some types of endings, whereas others are unaffected. The effect of ACh on the olfactory receptors has been studied by Skouby et al. (1954) who found that topical application of ACh produced an increased olfactory sensitivity. However, the observation that anticholinesterases do not block the activity of the olfactory receptors (Ottoson, 1962) indicates that ACh does not participate in the excitatory process. Whatever intermediary process might be involved, the ultimate effect of the odorous particles on the receptors is a reduction of their membrane potential. This change is the first step in the excitatory process that is amenable to direct examination.

The electrical response evoked when a small volume of odorous air is passed over the mucosa consists of a purely monophasic negative potential with a fast rising phase and an exponential fall (Fig. 1). This response is the generator potential of the olfactory organ and is homologous to potentials of the same type recorded from other sense organs. The configuration of the potential is influenced by a number of factors such as the parameters
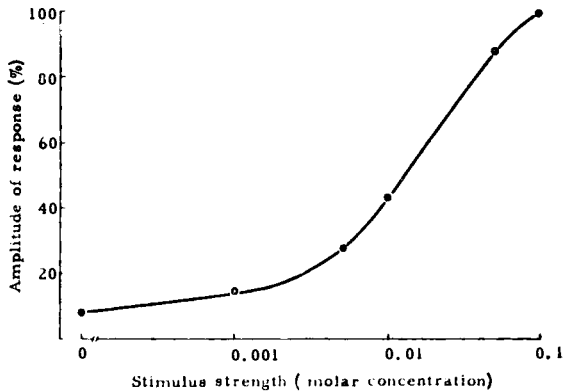
FIG. 2. Relation between amplitude of the electro-olfactogram and the stimulus strength. (From Ottoson, 1956.)

of the stimulus and the local conditions in the mucosa. In the frog, an air puff of a duration of about 1 sec. and of low or medium stimulus strength gives rise to a response that lasts for 4 to 6 sec. Compared with the corresponding potentials in other types of sense organs, the response of the olfactory membrane is relatively slow. This is to be expected, considering the fact that the exciting odorous particles have to pass through a layer of mucus before they reach the receptors. Excitation may therefore be assumed to take place gradually, its time-course being a function of the number of particles which reach the receptor per time unit. The temporal course of the excitatory process also depends on what may be called the " lifetime " of the odorous particles, i.e. the time that passes until they become inactivated.

The potential recorded from the sensory epithelium in the nasal mucosa is a mass response and as such it tells us very little about the function of the individual receptors. Nonetheless the response may provide information that might be useful for the evaluation of the function of the peripheral olfactory apparatus. By using the potential as an index of the activity of the olfactory membrane, the effect of different substances can be quantitatively measured. This method has been particularly valuable in studies on the relation between the physicochemical properties of different odorous compounds and their stimulating effects (Ottoson, 1958). By measuring the increase in amplitude of the response with increasing

stimulus intensity it has further been possible to evaluate the stimulus–response relationship of the olfactory organ (Fig. 2).

It is a common experience that the duration of the olfactory sensation varies for different substances. Some produce a short-lasting effect, while others give rise to a more persistent sensation. A comparison of the responses evoked by different substances of equal stimulus intensities shows that they may differ considerably in duration (Fig. 3). No systematic
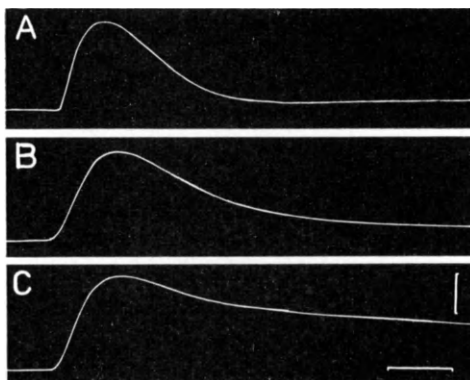
FIG. 3.   Responses to different types of stimuli.   A, amyl acetate ; B, butanol ;
C, oil of cloves.   Vertical line 1 mV.   Time bar 1 sec.   (From Ottoson, 1956.)

study has as yet been made about the relation between the time-course of excitation in the olfactory membrane and the properties of the stimulating substance. It seems most likely, however, that the solubility properties and the vapour pressure of the stimulus are of decisive importance with respect to the onset and duration of the response. It is of particular interest to note that corresponding differences have also been demonstrated in the time-course of the discharge in the secondary pathways (Adrian, 1951). These observations show that the temporal characteristics of the response are encoded in the pattern of the impulse discharge carried to the olfactory cortex and may serve an important function in the discrimination of different substances.

The notion seems to be widely held that the olfactory receptors adapt rapidly. However, this view is not supported by the results obtained in electrophysiological studies. In recording the olfactory discharge of the secondary neurons in the bulb of the rabbit, Adrian (1950) found that each inspiration of odorous air was followed by a distinct burst of impulses with no appreciable decrease of the activity for a period of 1 hr. or more. This observation clearly showed that the receptors are able to maintain their activity during prolonged periods of stimulation. Further evidence on this point has been obtained in recordings of the response of the olfactory membrane. As shown in Fig. 4, a potential with a sustained

plateau phase is produced when a continuous stream of air is passed over the olfactory mucosa. The level of the plateau in relation to the initial peak of the response is a direct measure of the adaptation of the receptors. With a stimulus of low or medium intensity this level amounts to 50–60 per cent of the height of the initial peak. The fact that this potential level is maintained with very little decline brings direct evidence that the olfactory receptors have to be classified as comparatively slowly adapting end organs. This is also evidenced by the slow decline of the response during
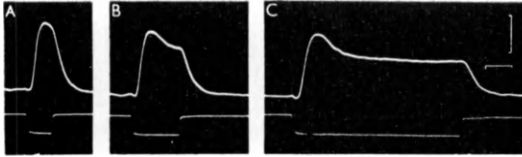
FIG. 4. Adaptation of the olfactory receptors. Responses obtained from the rabbit's nasal mucosa to stimulation with a constant stream of odorized air. Duration of stimulation : A, 0.5 sec. B, 1 sec. C, 3 sec. Vertical line 1mV. Time bar 0.5 sec. (From Ottoson, 1959a.)

repeated stimulation of the nasal mucosa. How are we then to explain the fact that an odour that at first appears strong rapidly weakens and soon becomes imperceptible? Adrian (1950) has suggested that this phenomenon may be explained by the fact that the incoming signals are suppressed by the intrinsic activity of the bulb. It is also likely that the efferent system (Kerr and Hagbarth, 1955) participates in this inhibitory action. Even if we accept this explanation, we are still faced with the difficulty of explaining the regularity of the adaptive process.

It has been demonstrated that the potential recorded from the olfactory membrane is not affected by antidromic stimulation of the olfactory nerves. This fact shows that the membrane producing this potential does not participate in the impulse activity. The potential generated by the receptors spreads electrotonically in the nerves and by reducing their membrane potentials initiates the afferent discharge. It has been demonstrated in studies on the impulse initiation in the lobster's stretch receptors (Edwards and Ottoson, 1958), in spinal motorneurons (Coombs et al., 1957), as well as in the giant cells of Aplysia (Tauc, 1962), that the cell body does not participate in the production of impulses. These findings may also apply to the olfactory primary neurons. If this view is correct the impulse initiation in the olfactory fibres would occur through the electrotonic spread of the potential generated by the hairs to the olfactory rod and cell body and further out in the axon where the impulse would be set up at the portion with the lowest threshold.

The extreme fineness of the olfactory fibres has been the major obstacle to the study of the characteristics of the afferent inflow and particularly to the successful analysis of the activity in single units. Some of the pro-

perties of the fibres, such as their conduction velocity and excitability cycle may, however, be examined by recording the activity in response to electrical stimulation. Amphibians and certain fishes are particularly useful preparations for such studies since the olfactory fibres in these species are grouped together into one single nerve trunk. Gasser (1956) has demonstrated that the spike potential of the pike's olfactory nerve consists of one single wave. The response of the frog's olfactory nerve (Fig. 5) has the
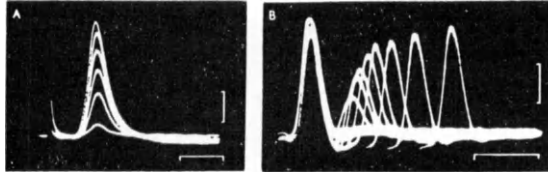
FIG. 5. Action potential of the frog's olfactory nerve. A, superimposed records of responses to electrical stimulation of increasing strength. Vertical line 0.5 mV. Time mark 50 msec. B, superimposed records showing the relative refractory period of the olfactory nerve. Vertical line 200 μV. Time bar 100 msec (From Ottoson, 1959c.)

same characteristic appearance (Ottoson, 1959c). The simple configuration of these responses can be attributed to the fact that all the fibres have approximately the same diameter (Gasser, 1956 ; de Lorenzo, 1960). Owing to their small dimensions conduction velocity is very low. In the pike, Gasser (1956) found the velocity to be about 0.2 msec. In the frog, the olfactory fibres conduct at a velocity of 0.14 msec. These findings indicate that time is of relatively little importance in the transmission of the olfactory message to the brain. In providing the olfactory cortex with information about the chemical environment, the absolute sensitivity of the olfactory receptors and their ability to function as peripheral analysers are most certainly far more important.

The transmission of the olfactory signals from the afferent fibres to the secondary neurons takes place in the glomeruli in the bulb. For the understanding of the function of the olfactory system it is important to note that there are no synaptic connections between the sensory cells in the mucosa. An interaction between the primary cells, resulting for instance in an inhibition of the type seen in the Limulus eye (Hartline et al., 1956) is therefore less likely to occur. Each receptor cell with its axon functions as an independent input channel to the bulb.

As shown in Fig. 6, natural stimulation of the olfactory mucosa gives rise to a slow potential change in the bulb. Superimposed upon this potential there are regular oscillations which in the frog have a frequency of 8–12 per sec. The experimental evidence suggests that the slow potential comes from the dendritic network in the glomeruli and that it is of the same nature as the enduring potentials recorded from sensory cortical areas

(Arduini *et al.*, 1957).   The induced waves are blocked by antidromic stimulation of the secondary olfactory pathways and are therefore most likely to be attributed to activity in secondary neurons.   There is a close resemblance between the potential developed in the olfactory membrane
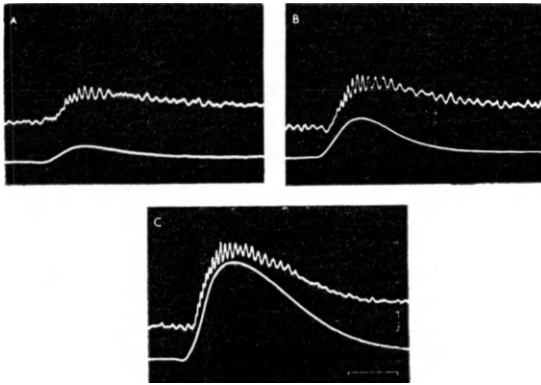
FIG. 6.   Simultaneous recordings of the electro-olfactogram and the bulb response.   Vertical bars 1 mV.   Time mark 1 sec.   (From Ottoson, 1959b.)

and the slow response of the bulb (Fig. 6).   This is of particular interest as the bulb response may be regarded as of the generator type.   As such it forms the link between the incoming signals and the impulse message carried to the olfactory cortex.   Information about the functional properties of the bulb has also been obtained in studies of the response produced by electrical stimulation of the olfactory nerve fibres.   The response developed by the bulb at the arrival of a synchronous volley of afferent impulses consists of a negative potential with a duration of about 150 msec. This response (Fig. 7) is composed of two separate components which may
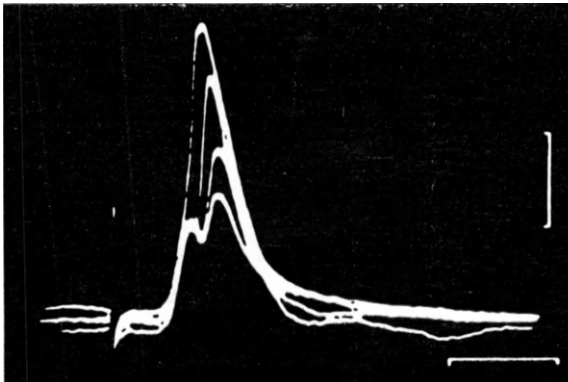
FIG. 7.   Response of the olfactory bulb to repetitive electrical stimulation (1 sec) of the olfactory mucosa.   Vertical line 1 mV.   Time mark 100 msec.   (From Ottoson, 1959c.)

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be segregated under various experimental conditions. Thus it has been demonstrated that the first component builds up a persisting potential during repetitive stimulation (Fig. 8), whereas the second component is
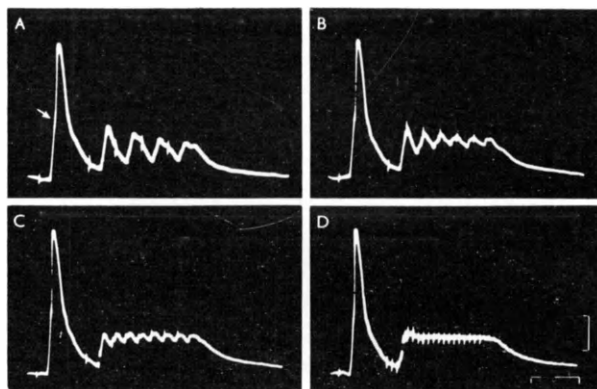
FIG. 8. Summation of the initial phase of the bulb response. Repetitive stimulation : A, 10 ; B, 15 ; C, 20 ; D, 50/sec. Vertical bar 1 mV. Time mark 200 msec. (From Ottoson, 1959c.)

unable to follow stimulation frequencies above 1 per sec without being considerably reduced in height. In has therefore been concluded that the first component is of synaptic origin, while the second one seems to reflect the activity in the secondary pathways. If this view is correct the first component would correspond to the slow potential of the bulbar response evoked by natural stimulation, while the second component would be

FIG. 9. Structural organization of the olfactory bulb. (From Van Gehuchten and Martin, 1891.)

produced by the same structures that give rise to the induced waves.

The structural organization of the synaptic connections between the primary olfactory fibres and the secondary neurons exhibits some interesting features (Fig. 9). The presynaptic endings make contact exclusively with the dendrites of the postsynaptic cells and not with their cell bodies. The first synapse in the olfactory system therefore represents a purely axo-dendritic interneuronal contact. Such a form of synaptic connection is typical of the nervous system in invertebrates and in some lower vertebrates. In higher vertebrates the majority of the synapses in the central nervous system are axo-somatic, i.e. the presynaptic fibres make direct contact with the cell bodies of the next neurons. This form of connection is considered to provide possibilities for a more efficient and rapid transmission of impulses than the primitive axo-dendritic synapses, where the impulses in the postsynaptic units are set up without any direct action on the cell bodies. There seems further to be reason to believe that the transmission in an axo-dendritic synapse occurs through a graded depolarization that spreads electrotonically along the dendrites towards the cell body. This implies that the signals are transmitted more slowly than in systems where the impulses are passed from one unit to another by axo-somatic connections. The unique organization of the olfactory synapse as represented by the densely interwoven nerve-nets of the glomeruli certainly also involves other functional characteristics. It is most likely that the arborization of the dendrites in the glomeruli has the function of collecting the incoming impulses, thereby securing the transmission of the olfactory signals. It is also possible that the glomeruli possess a functional specificity in the sense that fibres from receptors with similar sensitivity properties are directed towards particular glomeruli.

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OLFACTORY, VOMERONASAL AND TRIGEMINAL RECEPTOR RESPONSES TO ODORANTS

DON TUCKER*

SEVERAL years ago Beidler and Tucker (1955) discovered that one could record activity from the primary olfactory nerve in response to odorous stimuli. Typically, a small strand of the nerve is freed and cut centrally so as to form a peripherally directed twig, which is then placed on two Pt–Ir wire electrodes. Ringer's solution is replaced with mineral oil and differential recording employed. It is true that considerable practice is required for successful dissection. But for those who use electrical recording techniques there is an easier method that will demonstrate the nature of the activity recorded. One must remove the investing membranes from a small portion of the bulk of the nerve in a location that permits the physiological solution to be drained off and replaced with oil. Contacting the exposed surface of the nerve with a small electrode often yields surprisingly good records. Alternatively, the conducting solution may be left in place and the surface of the nerve contacted or penetrated with a small wire that is insulated except at the tip (Mozell, 1961). As might be expected, however, twigs raised up into oil give a higher signal-to-noise ratio and are stable over long periods of time.

I would like to mention questions that our research has raised and to submit incidental observations rather than to concentrate on polished results. My hope is that we can encourage electrophysiologists who specialize on the bulb or the nerve or the mucosa to examine the other recording sites, for general familiarity on the part of all will surely work toward our collective benefit. We may note that the major part of this symposium is devoted to gustation and that the taste receptor cell is distinct from the synapsing nerve fiber. There is a tendency to regard the olfactory receptor and nerve fiber as being unique because they are part and parcel of the same cell. But this is also true of the vomeronasal and trigeminal sensory systems. These three nasal sensory systems will be discussed for the domestic rabbit and a land tortoise known as *Gopherus polyphemus*.

TORTOISE

The head of a tortoise preparation is shown in Fig. 1, which is to orient the openings shown in greater detail in Fig. 2. In the trephined opening the main and accessory olfactory bulbs and the intracranial portions of the
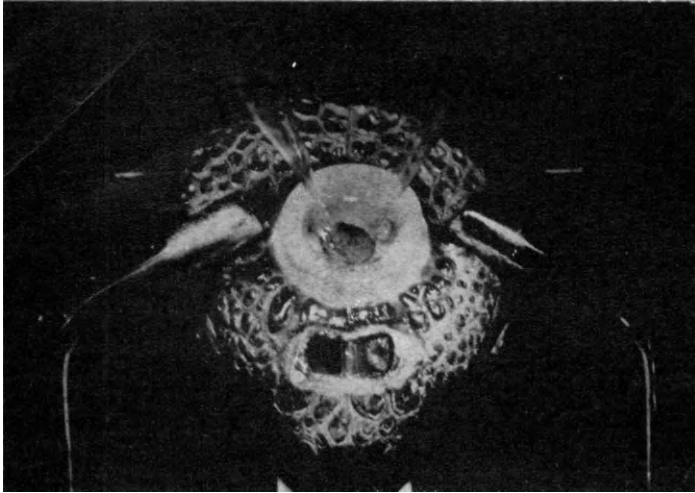
Fig. 1. Experimental arrangement for neural recording. The view of the tortoise's head is from above and in front.

olfactory and vomeronasal nerves are visible. The cut edges of the dural and pial membranes may be seen also. In the nasal exposure strands of olfactory nerve are prominently visible through the intact cartilage of the nasal capsule. Olfactory and vomeronasal twigs may be picked up intracranially, but trigeminal twigs must be got from within the capsule. For this purpose the trephine opening is advanced to overlap the rear of the nasal cavities. A prominent branch of the trigeminal nerve enters the nasal capsule ventrolaterally to the olfactory nerve and then curls up around it, splaying out in numerous branches distributed to the dorsal and medial nasal mucosa and to tissues external and anterior to the capsule.

Basic Techniques

Twigs from the medial part of the olfactory nerve project to the septal mucosa. With a window to the olfactory cavity as in Fig. 1, a stimulating electrode may be introduced and the mucosa explored for responses in a twig on the recording electrodes (Fig. 3). Alternatively, the intracranially situated twig may be stimulated electrically and the distribution of the antidromic compound action potential mapped on the mucosa. The olfactory receptors associated with a small twig of the nerve often fall in a slightly elongated area of less than one square millimeter.
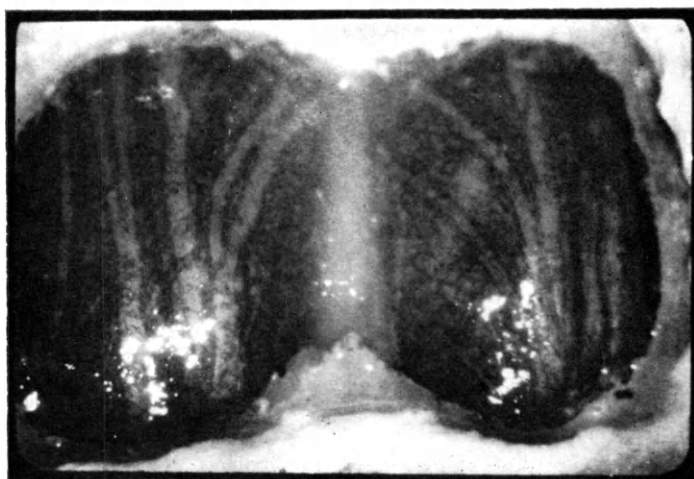
FIG. 2. Cranial and nasal exposures. The diameter of the trephined hole is 12–13 mm. The transverse dimension of the nasal exposure is about 9 mm.

Most often the nose is left intact for experimentation with odorous stimuli. In general, it is difficult to recognize discrete units in the activity
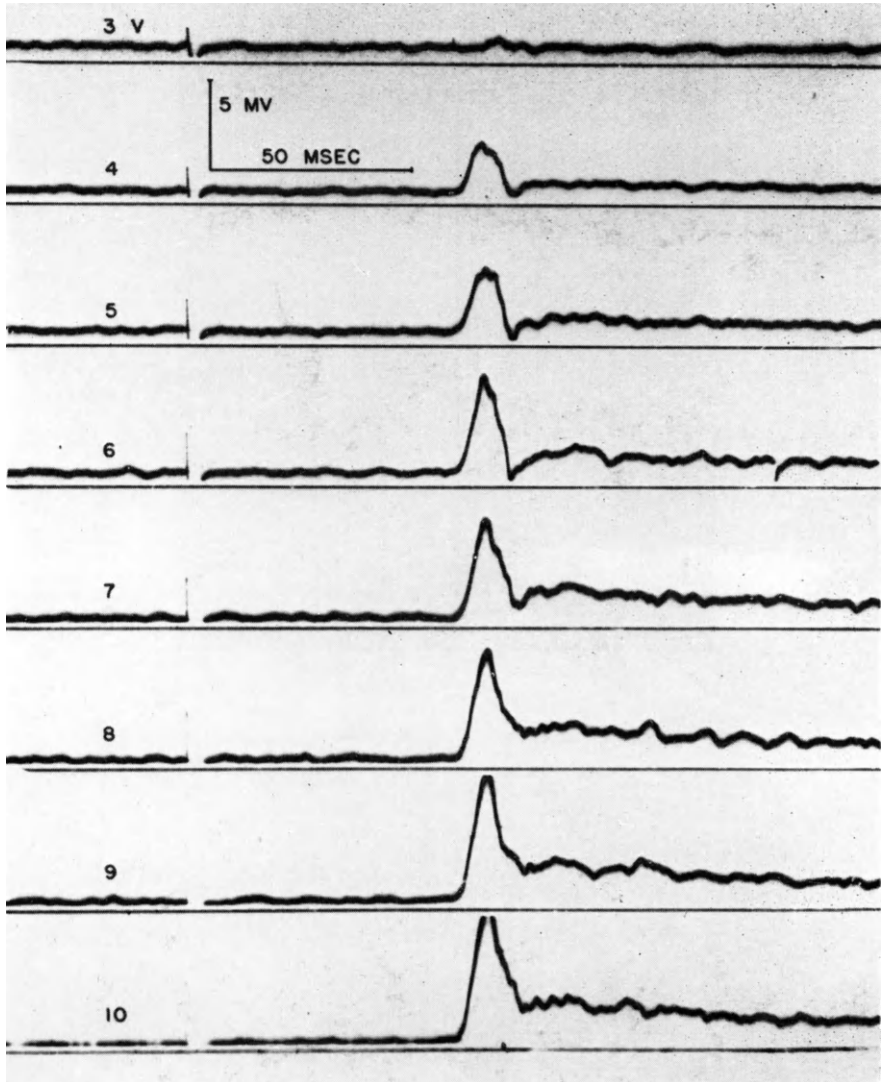
FIG. 3. Compound action potentials recorded from a twig of olfactory nerve in response to electrical stimulation of the olfactory mucosa. Differential recording with d.c. amplifier.

recorded from olfactory and vomeronasal twigs. But if the twig is sufficiently small and the background activity is not too high, one can get results such as are shown in Fig. 4. Nineteen hours later the preparation

had deteriorated sufficiently for the response to butyl acetate to appear as in Fig. 5. There seems to be no way to determine whether discrete activity

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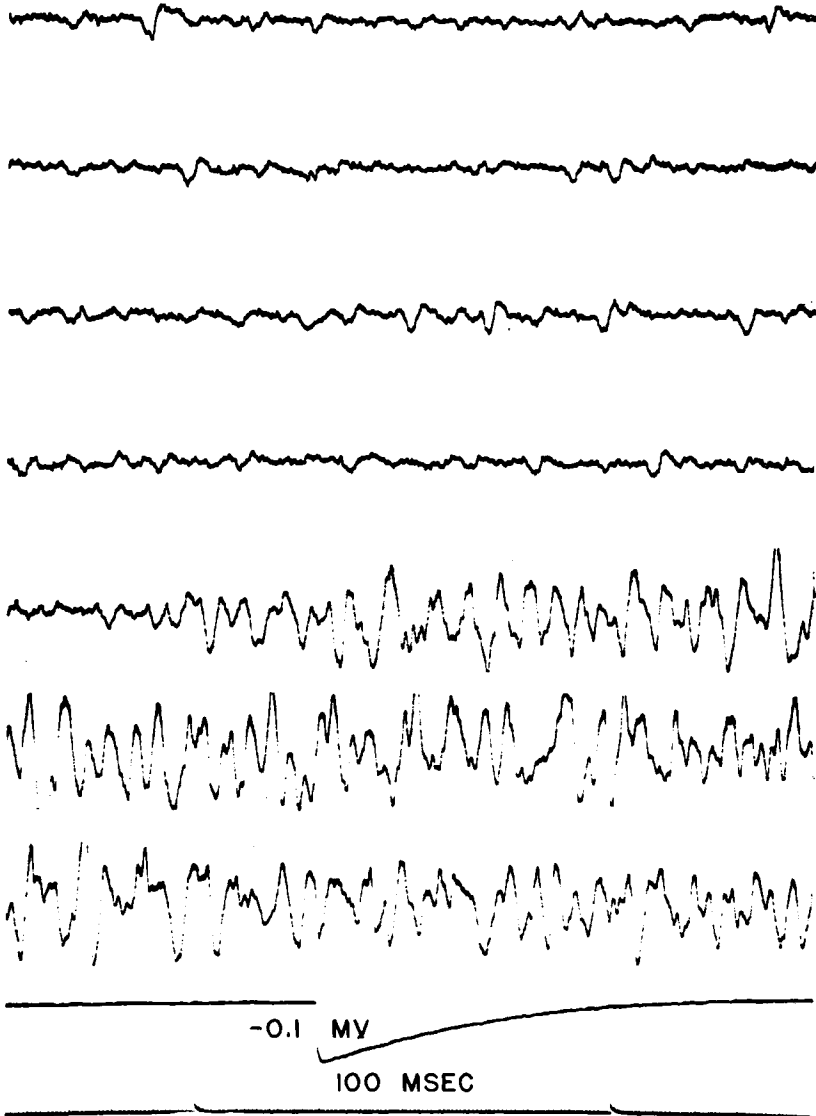
FIG. 4. Continuous record of discrete activity and response to butyl acetate obtained from an olfactory twig of diameter estimated at 12–15μ.

of this nature represents impulses in individual fibers or possibly groups of fibers acting in concert (Adrian, 1956).

Ten microns in diameter is about the lower limit of size for twigs to be handled readily and some even larger than 100μ yield good records. In any

event, narrow bandwidth amplification is useful for maximizing the signal-to-noise ratio. The fidelity of reproduction is low as a consequence, but
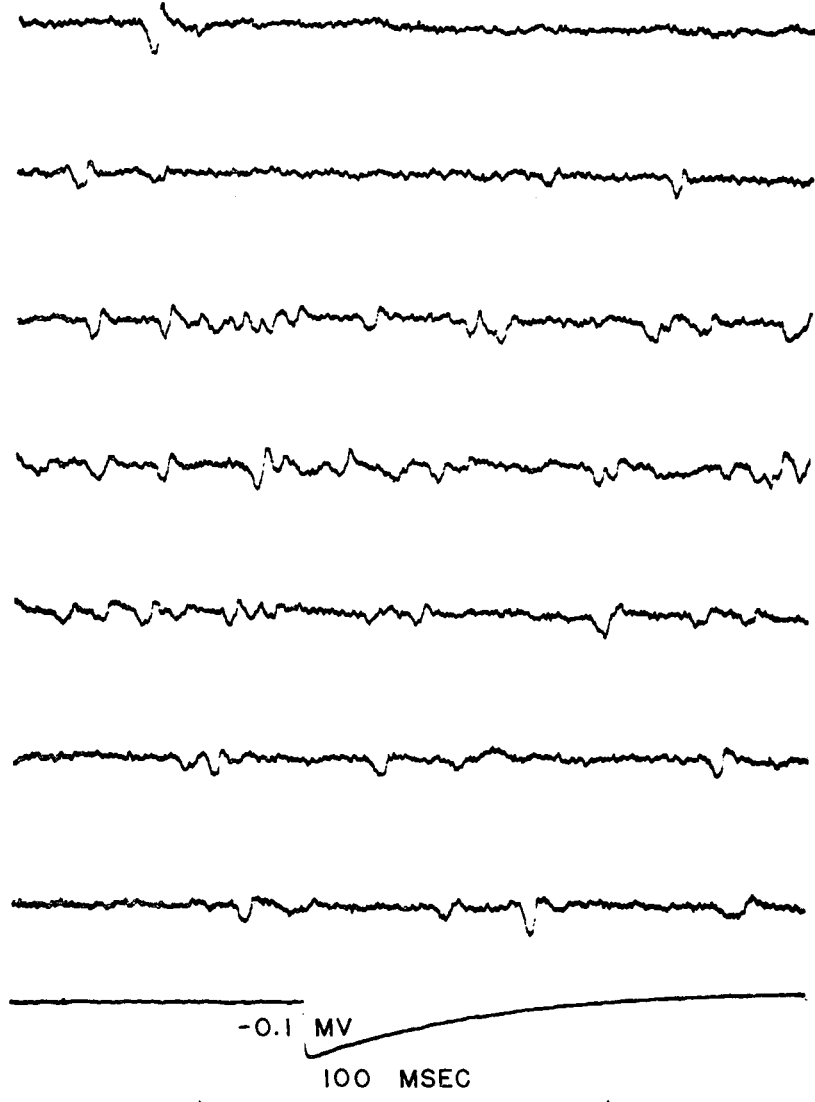
FIG. 5. Response to butyl acetate recorded 19 hr later from the preparation of Fig. 4.

little is gained by extending the upper frequency limit because of the increase in noise. Records obtained (a.c. and d.c.) simultaneously from an olfactory twig estimated to be 30μ in diameter are shown in Fig. 6. The animal was tracheotomized and for each odor a brief puff of air was directed

into the naris from a polyethylene wash bottle containing the odorant. Superimposed on the " slow potential " recorded with the d.c. amplifier is the " asynchronous activity " recorded with the a.c. amplifier. It seems natural to conclude that the d.c. record represents summation of action
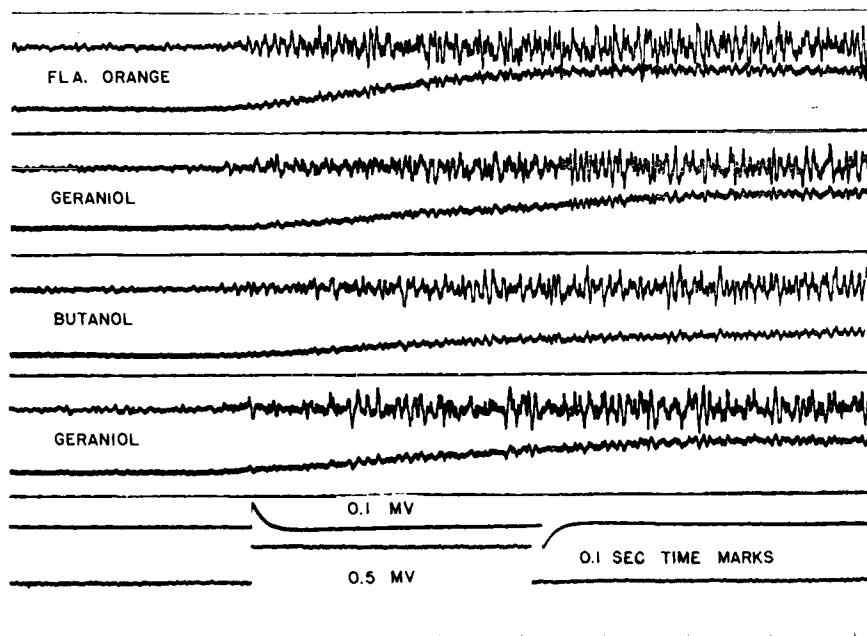
FIG. 6. Records obtained (a.c. and d.c.) simultaneously from an olfactory twig about 30μ in diameter. Both amplifiers were driven from one cathode follower connected to the recording electrodes.

potentials at the recording electrodes. A twig so small as 10μ in diameter could contain an upper limit of 2500 fibers uniformly 0.2μ in diameter (Gasser, 1956).

Recording of slow potentials from the olfactory mucosa is a technique that has become popular and for such records Ottoson (1956) has proposed the name electro-olfactogram. It is also possible to record asynchronous activity from the olfactory mucosa. However, the nerve is the best site for recording asynchronous activity and the mucosa appears to be the best site for recording slow potentials. An electrode similar in design to Ottoson's, except that polyethylene instead of glass tubing was used, was inserted through a small hole in the top of the nose and the residual opening closed with silicone high-vacuum grease. Air was flowed through the nose at constant rate and amyl acetate of various concentrations introduced. The d.c. record is shown in the first trace of Fig. 7 and the asynchronous activity after passage through Beidler's (1953) " integrator "

circuit is shown in the second trace. Positioning of the electrode tip is critical for recording asynchronous activity from the mucosa. A very small advance after the initial contact with the mucous surface is optimal, provided that the layer of mucus is thin.
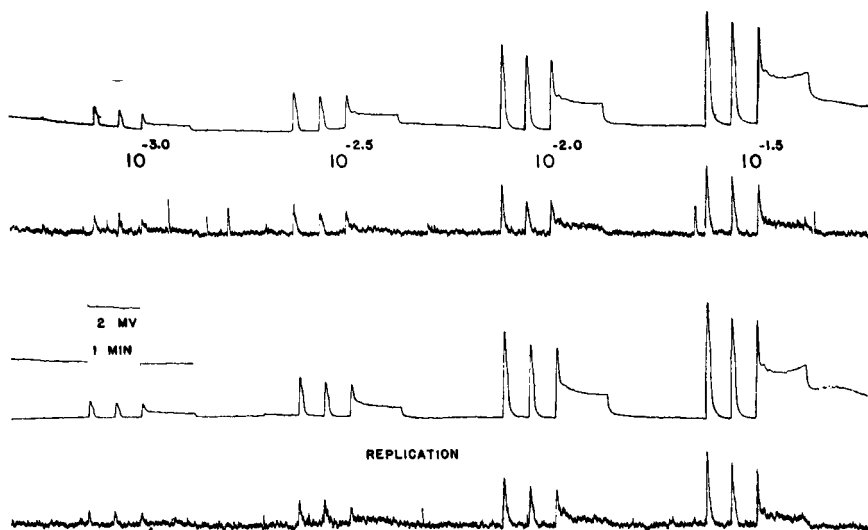
FIG. 7. Olfactory mucosal recording with Ag–AgCl-Cl⁻ electrode. The first trace is output from the d.c. amplifier recorded directly and the second trace is output from the a.c. amplifier after passage through Beidler's integrator circuit.

Many of our data are in the form of integrator records taken with a.c. amplification because they are easily obtained and quantified. Stimulus quantification is achieved with a dilution type olfactometer to vary concentration of odorant, a system to control nasal flow rate and a switching mechanism to alternate between clean and odorous air independently of nasal flow rate (Tucker, 1962).

Olfactory-Vomeronasal Comparison

It was a surprise to find the vomeronasal receptors in the tortoise responding to odorants as readily as do the olfactory receptors. There would appear to be no doubt about identification, for the termination of the vomeronasal nerves in the accessory olfactory bulbs is definitive, as may be seen in Fig. 2. Besides, one can readily distinguish between the two kinds of receptors after a little practice by testing with selected odorants. This is conveniently done with the puff technique of stimulation, using wash bottles containing small quantities of the odorants. The intensity of stimulation is varied by controlling the strength of the squeeze and the distance between the tip of the nozzle and the naris.

The basis for comparison in simultaneous recording experiments is

adjustment of the stimulus to yield moderate responses from either or both kinds of receptors. The vomeronasal response tends to predominate for the lower members of a homologous series of compounds. It is larger for methanol and ethanol as shown in Fig. 8 and for all the succeeding alcohols
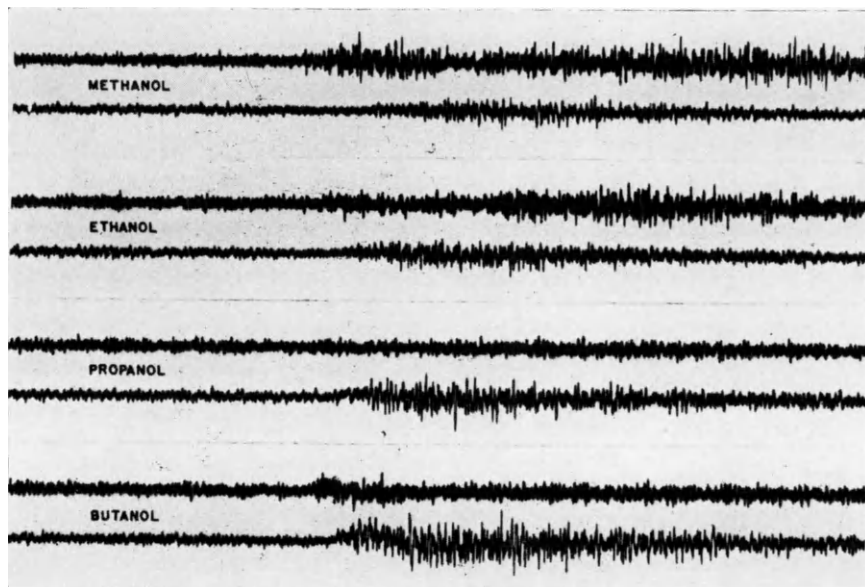
FIG. 8. Vomeronasal (first trace of each pair) and olfactory responses recorded simultaneously. Differential nature of the responses is illustrated.

the olfactory response is larger. In the fatty acid series the cross-over from vomeronasal to olfactory predominance occurs in going from *iso*-valeric, after *n*-butyric, to *n*-valeric acid. The olfactory response is larger for the acetate esters of all the aliphatic alcohols.

The vomeronasal organ, or organ of Jacobson, in *Gopherus* opens into the nasal cavity via a tubular duct that is relatively small. One might expect that the olfactory receptors should be more readily stimulated, but no consistent difference in latency between responses of the two kinds was seen (Fig. 9). How can odorant molecules penetrate to the sensory epithelium lining the organ of Jacobson as readily as to the much more accessible olfactory organ ?

The vomeronasal organ is housed in a projection from the wall of the nasal septum. This projection has the appearance of a baffle, such that the respiratory passageway is deflected laterally before descending on its route to the choana in the roof of the mouth. The histological section shown in Fig. 10 is through the organ at a level where the vomeronasal nerve is breaking up into branches. Notice the proximity of the epithelial surface

at the posterior boundary of the nasal vestibular chamber. Although this problem is still unresolved, I believe it likely that the vomeronasal nerve innervates this highly accessible patch of tissue in the anterior part of the nasal cavity. Actually, it is probably doubtful that receptors in the organ of Jacobson proper were ever stimulated in these experiments.
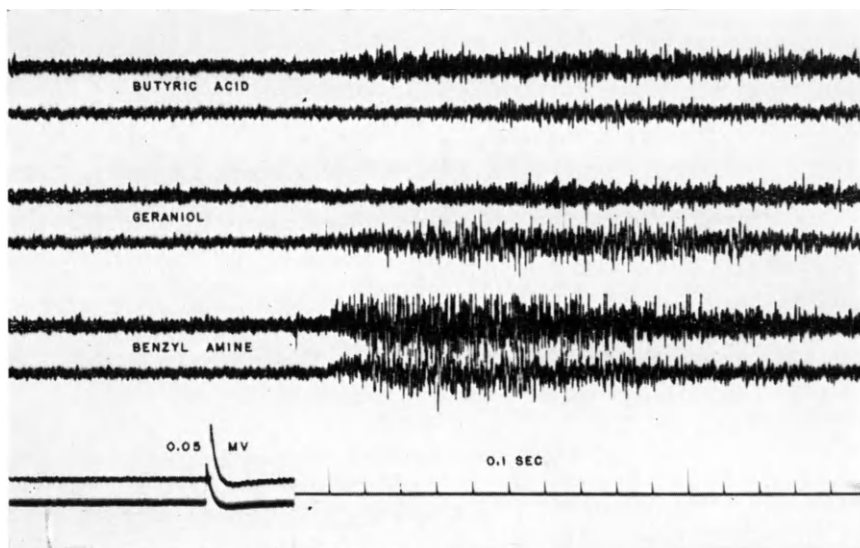
FIG. 9. Same preparation as for Fig. 8. Inconsistent difference in latency between vomeronasal (first trace of each pair) and olfactory responses is illustrated.

Olfactory Study in Detail

An apparent difficulty in the use of cold-blooded animals under anesthesia is the paucity of active respiratory movements. We tested tricaine methanesulphonate (MS 222—Sandoz), but it was difficult to keep the animal at such a level of anesthesia that respiration was frequent but that no struggling occurred. Figure 11 shows integrated olfactory responses occurring during free respiration. Each increase in response coincides with inspiration. The response is a function of the rate and depth of inspiration, which can be vexing when the animal's breathing is irregular.

Most experiments were performed with the animal tracheotomized and under ethyl urethane anesthesia. The choana was cannulated and air was drawn through the nose, which was fitted into a port in the side of a cylindrical glass breathing chamber. Air flowed into the breathing chamber at a constant rate of $100 cm^3/sec$. That which was not drawn into the naris passed the tip of the nose at right angles and exited freely to atmospheric pressure. A flow-splitting system was arranged to switch between

odorous and clean air without altering the flow rate into the breathing chamber.

(a) *Relative humidity, gaseous medium, and temperature.* Olfactory responses to amyl acetate in the concentration range of 10^{-3} to 10^{-2} of

FIG. 10. Section through Jacobson's organ approximately parallel to the sagittal plane, stained with Gomori's trichrome. The naris is to the left. The maximum linear distance in the lumen of Jacobson's organ is about 1 mm. Heavily stained bundles of trigeminal nerve appear directly above the organ. Lightly stained bundles of vomeronasal nerve appear above the trigeminal nerve tissue and extend into the region between Jacobson's organ and the nasal vestibule.

saturation at 20°C were independent of relative humidity. An effect of dry air was to accelerate deterioration of the preparation, which was evident only at high nasal flow rates.

Nitrogen, oxygen, argon and mixtures thereof were tried in place of air as the carrier gas for amyl acetate. No effect on the phasic (initial) olfactory response was seen. Second-order effects on the tonic olfactory response to amyl acetate and background olfactory activity were seen upon exclusion of oxygen or introduction of carbon dioxide (Tucker, 1962).

Temperature variation over the range of 20-30°C had practically no effect. Amyl acetate concentrations were maintained in terms of saturation

at 20°C, but the temperatures of the preparation and of the gas delivered to the naris were varied together. Over the range of 10-35°C the integrated olfactory response increased slightly with decrease in temperature for a constant mole fraction of amyl acetate. The response in terms of nerve impulses per unit time might decrease with a decrease in temperature, since broadening of the impulses with cooling would cause greater individual contributions to the integrator record.
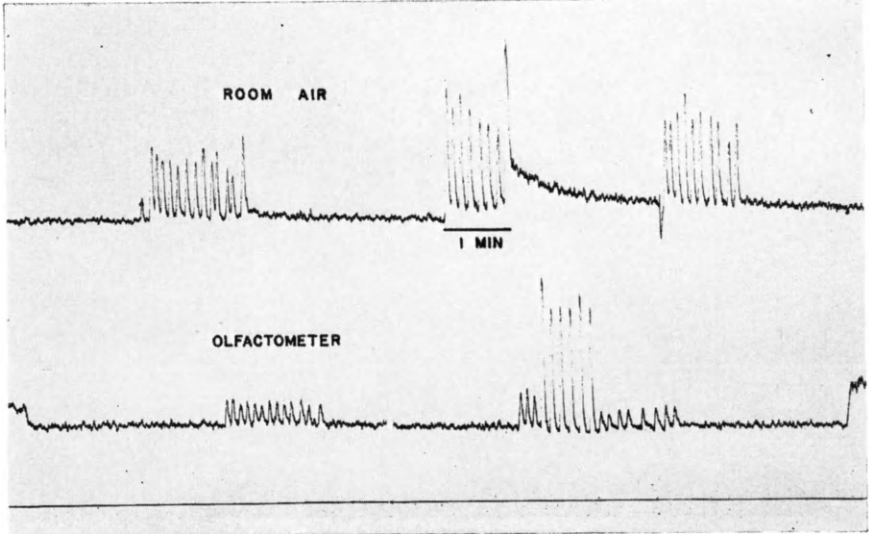
FIG. 11. Integrated olfactory responses recorded during free respiration, which was of intermittent nature. Between the first and second groups in the first trace a cigar was lit and smoke was blown at the animal near the end of the second group. In the second trace cleaned air and amyl acetate at $10^{-7/3}$ of air saturated with the odorant were delivered to the breathing chamber from the olfactometer.

(b) *Odorant, concentration, and nasal flow rate.* Odorant species, odorant concentration and volume flow rate of the odorous medium into the naris are the important parameters, among those which can be specified external to the nose, for determining the olfactory response. This is also true for the other nasal sensory systems that respond to odorants. Figure 12 illustrates flow rate and concentration dependence for the responses of two olfactory twigs to amyl acetate. The figure also introduces an intranasal parameter of importance for determining the response—position of receptors in the olfactory organ. It need hardly be pointed out that Adrian (1956, 1951) deduced from olfactory bulb recordings the variation of the stimulus over the organ. One may say that the accessibility of odorant molecules to the receptors is different for various locations in the organ. Matters are complicated further by the finding that olfactory accessibility can be varied by changes in the physical dimensions of the nasal cavities.

Phasic olfactory responses are graphed as a function of flow rate for two odorants in Fig. 13. Periodic testing with amyl acetate showed that the olfactory accessibility was considerably greater during collection of the benzyl amine data than it was for the amyl acetate run. Thus, the curves
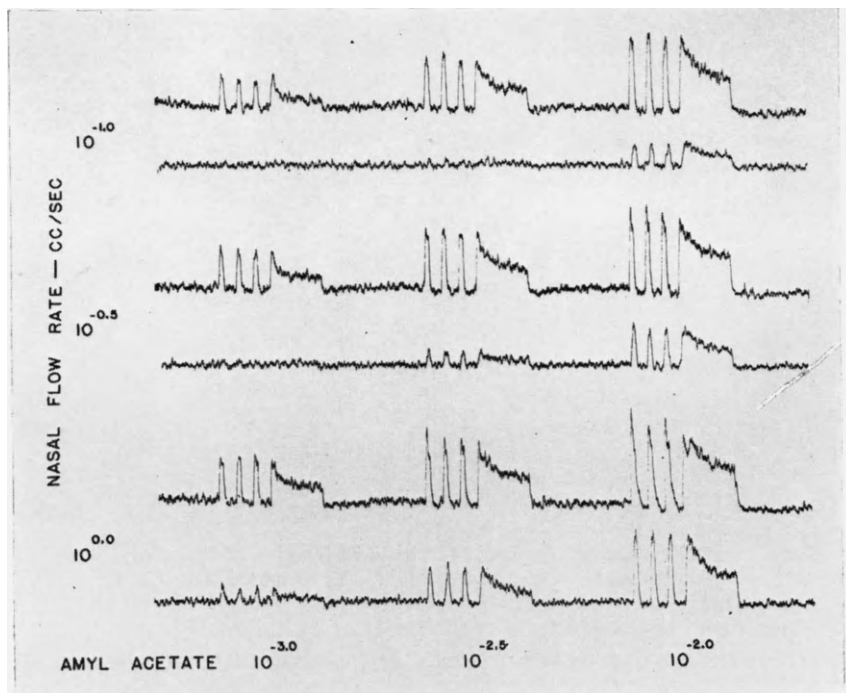
FIG. 12. Comparison of integrated olfactory responses, recorded simultaneously from two nerve twigs, for variation of nasal flow and concentration of amyl acetate. The unit of concentration is saturation at 20°C. Stimulation procedure was odor on 5 sec of every 20 sec followed by 1 min on.

for benzyl amine would be shifted to the right if the accessibility were reduced to the level that existed during the amyl acetate run. The maximal response to benzyl amine is sizably greater than that to amyl acetate. By contrast, the maximal response that can be obtained with butyric acid is much smaller than either of these. For a sufficiently low concentration of amyl acetate, the response as a function of nasal flow rate reaches a maximum that is less than the characteristic maximal value.

Plateauing of the olfactory response with nasal flow rate, in the range that is physically possible, appears to correlate with low aqueous solubility of odorants, although only a few have been examined. Response dependence on the flow rate clearly reflects the need to deliver odorant molecules to the receptor sites. The solubility relation suggests that plateauing is

caused by an approach to equality of odorant chemical potential in the medium of the receptor sites with that in the gaseous medium entering the naris, which is known. Since this discussion applies to the phasic olfactory response, the odorant thermo-dynamic activity specified at the receptors is
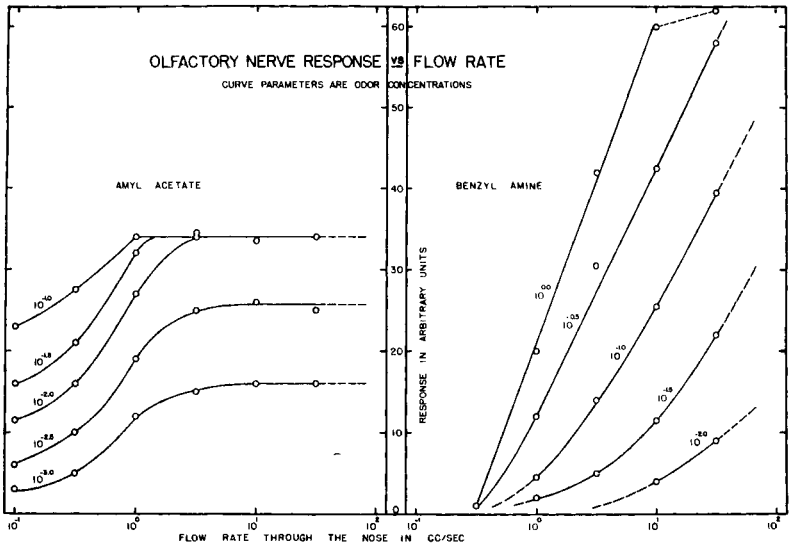
FIG. 13. Phasic olfactory responses graphed as a function of nasal flow rate for two odorants. Unit concentration is air saturated at 20°C.

that which is attained in a time of the order of a characteristic time inferred for initiation of the response. It may be possible, then, to relate the response of the receptor to the stimulus at the level of the receptor. Indeed, olfactory responses to amyl acetate taken in the range of flow rate for plateauing of the response were found to be quantitatively similar in different preparations. Response data as a function of concentration were fitted with Beidler's (1961) taste equation for two different sets of independent sites. The algebraic form of the equation is

$$r = \frac{R_1 \ K_1 \ [A]}{1 + K_1 [A]} + \frac{R_2 \ K_2 \ [A]}{1 + K_2 [A]}$$

and the transcendental form for the semilogarithmic plot is

$$r = \frac{R_1}{2} \ (1 + \tanh \tfrac{1}{2} \ln K_1 \ [A] + \frac{R_2}{2} \ (1 + \tanh \tfrac{1}{2} \ln K_2 \ [A],$$

where r_i is the response ($r = \Sigma r_i$)

R_i is the maximal value of response

K_i is the equilibrium constant

$[A]$ is the concentration of amyl acetate

i is the site index.

More correctly, one should use double-subscript notation to identify both the kinds of site and kinds of odorant. The numerical values found for the constants in the equation are

$$\frac{R_2}{R_1} = 2.5,$$

$K_1 = 17,800$ reciprocal concentration units

and $K_2 = \quad 274$ reciprocal concentration units,

where the unit of concentration is air saturated at $20°C$ with amyl acetate. It would be valuable if a way could be found to determine the absolute value of response in terms of nerve impulses per receptor and unit of time. Dr. Shibuya's current work in our laboratory may point the way to this goal.

(c) *Aqueous solutions of odorants.* Systematic study showed that the minimum requirements for a solution to approximate the normal mucous environment of the receptors is osmotic pressure about equal to that of the blood and the presence of calcium ions. The solution devised for *Gopherus* contains 1.4 mM $CaCl_2$ and 0.17 M NaCl or its osmotic equivalent of sucrose. Freshly made solutions invariably contained contaminating odorants. Slow percolation through activated cocoanut charcoal, previously equilibrated with the solution, reduced odorous contamination to tolerable levels. The subject of unwanted odors is highly practical and has not received the attention due it, but the whole of this paper could easily be devoted to the topic.

Olfactory receptors were found to be insensitive to variation of ionic strength, to variation of pH over several units and to variation of osmotic pressure over a range of about $\pm$ 20-25 per cent. Increase of calcium ion concentration to many times the 1.4 mM level had negligible effect on the background neural activity (contaminating odorants?), but with decrease below that level the activity rose rapidly. Solution free of calcium ions flowed continuously through the olfactory cavity initially causes intense receptor activity and after a few minutes the receptors are killed. The animal's freshly spilled blood is intensely stimulating to the olfactory receptors, but no harm seems to be done. Bleeding occurs when the opening to the olfactory cavity is made in the top of the nose (Fig. 1).

Aqueous solutions were introduced through the cannula in the choana and flowed out the window in the top of the nose. The naris was plugged loosely with cotton. Cleaned solution medium was used to make up various concentrations of odorants. Control apparatus was arranged to alternate the flow between solutions with and without added odorant. The records obtained in these experiments were remarkably similar to those taken with the same odorants introduced in the gaseous phase. Vomeronasal and olfactory responses recorded simultaneously are shown in Fig. 14. The

differential nature of the responses made by these anatomically similar
nerves to butyric acid and amyl acetate is quite evident. The tonic (steady
state) olfactory response is notable at the higher concentrations of butyric
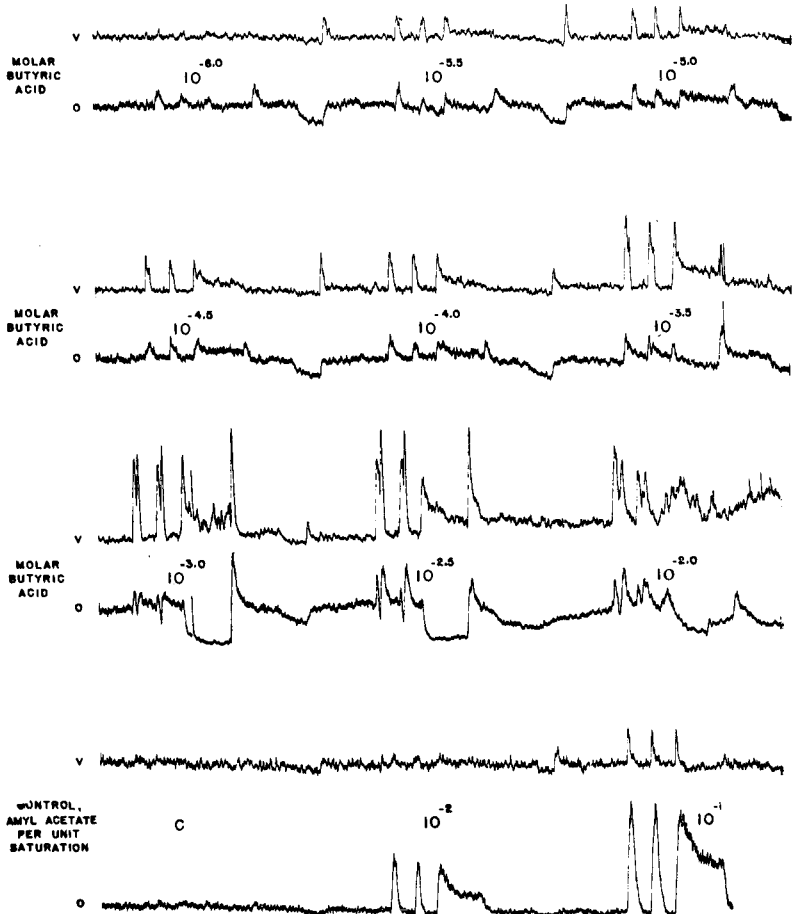
FIG. 14. Integrated vomeronasal and olfactory responses to odorants introduced
in an aqueous solution medium. Stimulation procedure: flow at 1/3 ml/sec of
cleaned solution was initiated. After about 1 min flow was switched to odorous
solution for 5 of every 30 sec twice and then for 60 sec.

acid. However, other odorants produce a similar though not so marked
depression at high concentrations. Both kinds of receptors, vomeronasal
and olfactory, developed injury discharges at the highest concentration of
butyric acid. Other odorants produce this effect too.

Trigeminal Receptors and Autonomic Effectors

Trigeminal receptors that respond to odorants appear somewhat more
similar to the vomeronasal receptors in the kinds of odorant to which they

respond readily, but amyl acetate is an exception. Although the vomero-nasal response to amyl acetate tends to be small, it appears shortly after the olfactory response with increase of concentration.   On the other hand, the trigeminal response has a well-defined threshold lying between $10^{-1.5}$ and $10^{-1.0}$ of amyl acetate at saturation.   This is one reason why amyl acetate has proved so useful in our work, for nearly all the reflex effects under anesthesia that we have seen appear to be stimulated by trigeminal activity. In the discussion of Fig. 13, it was noted that for the receptors being monitored, the olfactory accessibility was greater during the benzyl amine run than during the amyl acetate run.   The trigeminal response exhibited a strong flow rate dependence as did the olfactory response, but the trige-minal response appeared first either with increase in concentration of benzyl amine or increase of nasal flow rate.   We believe that the cause of the change in olfactory accessibility was due to changes in the nose pro-duced by reflexes in response to trigeminal receptor stimulation.   A similar change in olfactory accessibility was often seen for temperature in the range of 10-15°C.

An important mechanism for variation of olfactory accessibility is surely the changing of physical dimensions within the nasal cavities.   Such a change can be demonstrated by stimulating electrically the cervical sym-pathetic nerve in a preparation with a window in the top of the nose.   The size of the aperture between the vestibular chamber and the olfactory cavity proper is reduced markedly with activation of the sympathetic effectors.   The effect on the olfactory response to an odorous stimulus of constant parameters at the level of the naris is shown in Fig. 15.
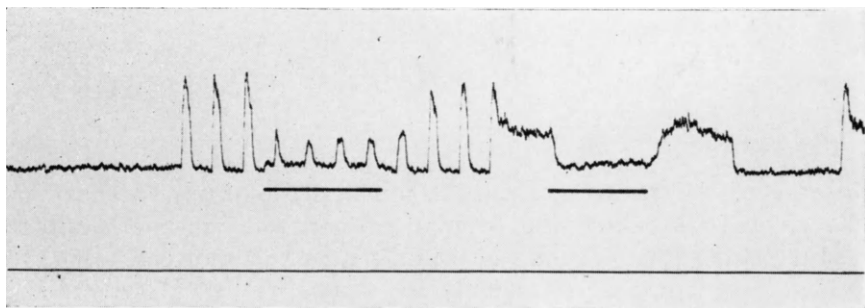
FIG. 15.   Reduction of phasic and tonic olfactory responses caused by electrical stimulation (bars) of the cervical sympathetic nerve.   Constant flow through the nose at 1 msec and amyl acetate concentration in air held at $10^{-2.5}$ of saturation.

Amyl acetate concentration was varied up through $10^{-1.5}$ of saturation for the records of the mucosal potential shown in Fig. 7.   The electrode invariably lost contact with the mucosa whenever $10^{-1.0}$ amyl acetate was

presented.   Thus it is now evident that movement of the mucosa relative to the tip of the electrode occurred.

Trigeminal activation is often accompanied by profuse flow of nasal secretions.   An increase in the rate of mucous secretion from the olfactory mucosa and in the rate of flow of mucus over it would conceivably reduce the accessibility of odorant molecules to the receptors.

RABBIT

Although we have invested much more work in studying the rabbit, the results are not so extensive for this animal as for the tortoise.   The anesthetized rabbit seems to be a comparatively poor preparation for recording from the primary olfactory nerve.   We believe that in large part this is
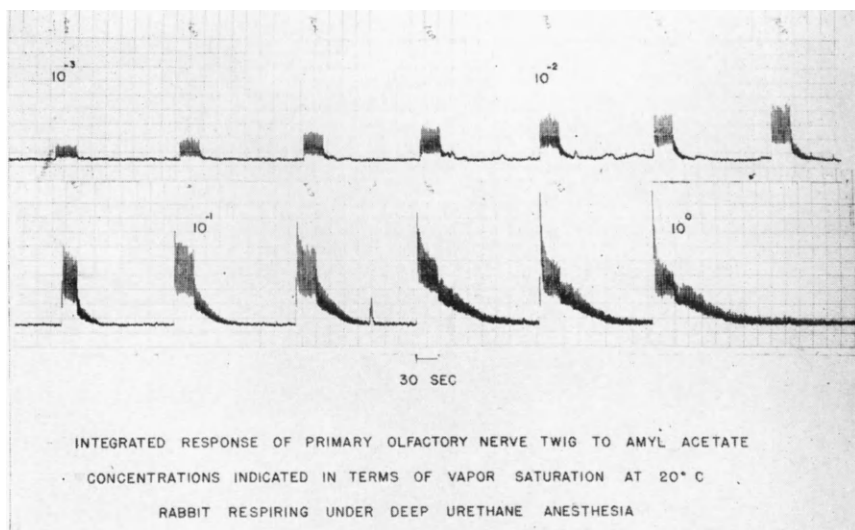
Fig. 16.   Rabbit olfactory response for amyl acetate concentration series in 1/4 log steps.

because of the greater geometrical complexity of the nasal anatomy. Hopefully, Dr. Moulton's (this Symposium) work with implanted electrodes will reveal the nature of some of the differences we can expect to find for rabbits in the conscious and anesthetized states.

Olfactory Receptors

Most olfactory twigs were picked up from the dorsomedial aspect of the nerve at the level of the cribriform plate.   These twigs project to areas in the mucosa high up on the septal wall and over onto the lateral arch. Fortunately, this seems to be one of the most accessible portions of the organ for olfactory stimuli.

Olfactory responses recorded during free respiration for a concentration

series of amyl acetate are shown in Fig. 16. The question is frequently
asked whether the olfactory receptors show adaptation, or is adaptation of
olfactory sensation largely a central nervous system phenomenon? The
answer is that phasic responses occurring with free respiration are main-
tained indefinitely for low levels of response, but for more intense stimula-
tion the responses decrease to a level which is maintained thereafter. Such
depressed levels of response can approach complete absence of activity
for some odorants. e.g. chloroform. However, for continuous stimulation
achieved with unidirectional flow of odorous medium through the nose,
the tonic response is always less than the phasic response. One presupposes
elicitation of the phasic response, for which it is necessary to attain the
stimulus concentration in a sufficiently short time. Figure 16 shows also
that after long exposure to a high concentration of amyl acetate there is a
considerable time required for the residue of odorant to fall below a detect-
able level.

A strong flow rate dependence of the rabbit olfactory response to amyl
acetate is shown by the records in Fig. 17. There is no evidence of plateau-
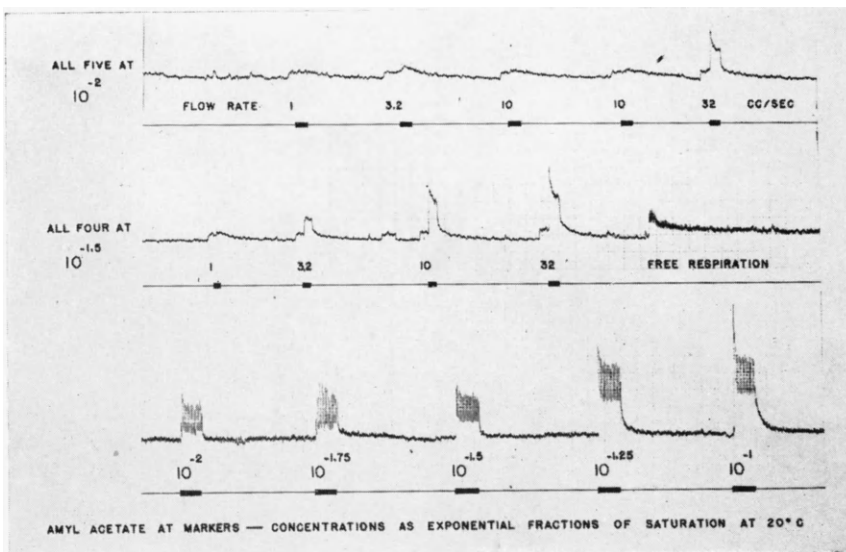
FIG. 17. Flow rate dependence of rabbit olfactory response.

ing of the response as was typical for the tortoise. But it is difficult to
flow air through the passive nose of the rabbit at rates approaching the
higher values we believe are often attained momentarily during free respira-
tion. Perhaps with introduction of odorous gas through a surgical window
the flow rate dependence can be reduced sufficiently to get data suitable for
curve fitting.

Vomeronasal Receptors

The vomeronasal system of the rabbit has proved to be a knotty problem. Usually there is no sign of response to odorants, yet in some circumstances there is much variation of vomeronasal neural activity that often appears to be spontaneous. Preparations are highly individualistic. A procedure that serves to modify the activity in one is more often than not without effect in another. Electrical stimulation of the cervical sympathetic nerve, temporary occlusion of the pharynx with finger pressure applied above the larynx, probing of the anterior palate and cannula insertion in the canal of Stenson frequently cause changes in the activity. Spontaneous licking, chewing and swallowing movements under light anesthesia are commonly accompanied by fluctuations of vomeronasal receptor activity. Changes that do occur with introduction of odorous air in the naris seem usually to be produced reflexly and not to be direct responses of vomeronasal receptors to the odorant.

However, the records of Fig. 18 contain examples of what I believe are
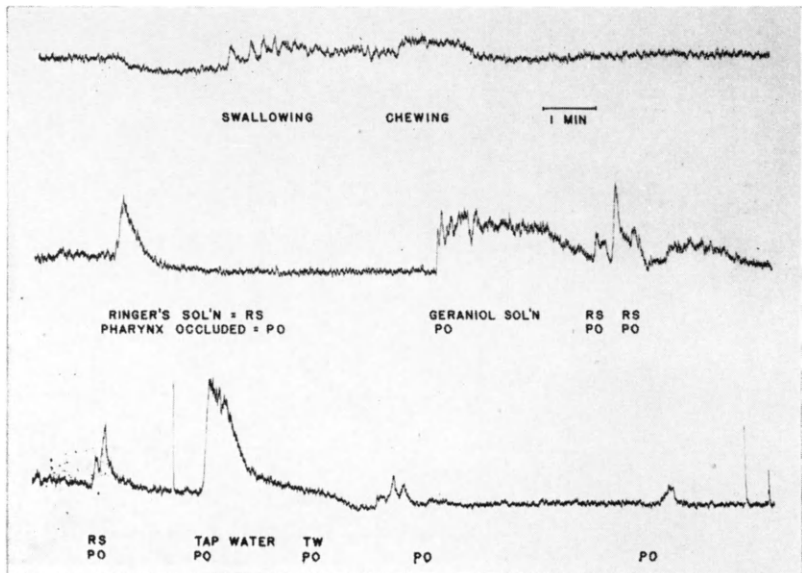
FIG. 18. Integrator records of activity recorded from rabbit vomeronasal nerve. Solutions were introduced from the canal of Stenson in sufficient quantity to appear at the naris.

responses to odorants in aqueous solution. On the basis of experience with such solutions in the tortoise, the responses with Ringer's solution (contaminating odorants), geraniol and tap water (lack of Ca^{++}) seem to be genuine. Through a cannula inserted a few millimeters into the canal of

Stenson 0.3 ml of solution was introduced, which was sufficient quantity to cause its appearance at the naris, and then the pharyngeal occlusion maneuver was performed.   Hamlin's (1929) suggestion of emptying and filling of Jacobson's organ may be appropriate to this case.

A curious finding was that with replenishment of mineral oil over the cranial exposure a temporary increase in vomeronasal activity ensued. Variation of temperature of the added oil showed that local cooling was the cause.   The oil over the brain obviously equilibrates at some temperature above that of the room.   The effect was obtained with active olfactory and trigeminal twigs, from which it was seen that cooling at the recording electrodes causes greater duration of impulses (Fig. 19), and consequently,
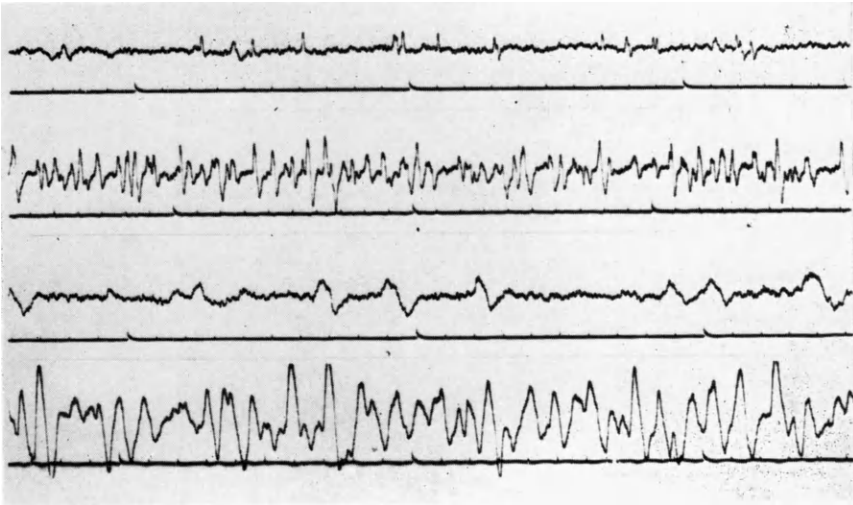
FIG. 19.   Oscilloscope traces of sensory activity in ethmoidal nerve twig taken before and during stimulation with heptyl alcohol ; at temperature equilibrium of the oil bath for the first pair and after addition near the electrodes of 2–3 drops of oil at 10–12°C for the second pair.   Time marks at 10 and 100 msec.

a greater output from the integrator.   The apparent greater sensitivity of the vomeronasal nerve to local cooling probably means that background activity of these receptors is high, at least for the conditions of the experiments.   Perhaps the vomeronasal receptors are performing in their function without our recognizing the fact.

Trigeminal Receptors

Oscilloscope tracings of trigeminal receptor activity before and during presentation of heptyl alcohol are shown in Fig. 19, which demonstrates also the effect of cooling at the recording electrodes.   In general, there is some background activity, tending to synchronize with inspiration, for

presentation of clean air from the olfactometer. It is not known whether this represents chemical, thermal, mechanical or other types of stimulation of trigeminal receptors. However, for obvious mechanical stimulation the individual action potential spikes are of much greater amplitude.

The threshold concentration of amyl acetate for the rabbit's trigeminal response falls in the same range as for the tortoise, namely $10^{-1.5}$ to $10^{-1.0}$ of saturation at 20°C. We have not yet found another odorant with as large a spread between the olfactory and trigeminal threshold concentrations. In fact, the trigeminal response probably appears first for the smaller members of the aliphatic alcohol series. Integrated trigeminal responses to alcohols are shown in Fig. 20 for the relatively crude stimulation technique of free respiration into the mouth of a 125 ml Erlenmeyer flask containing a
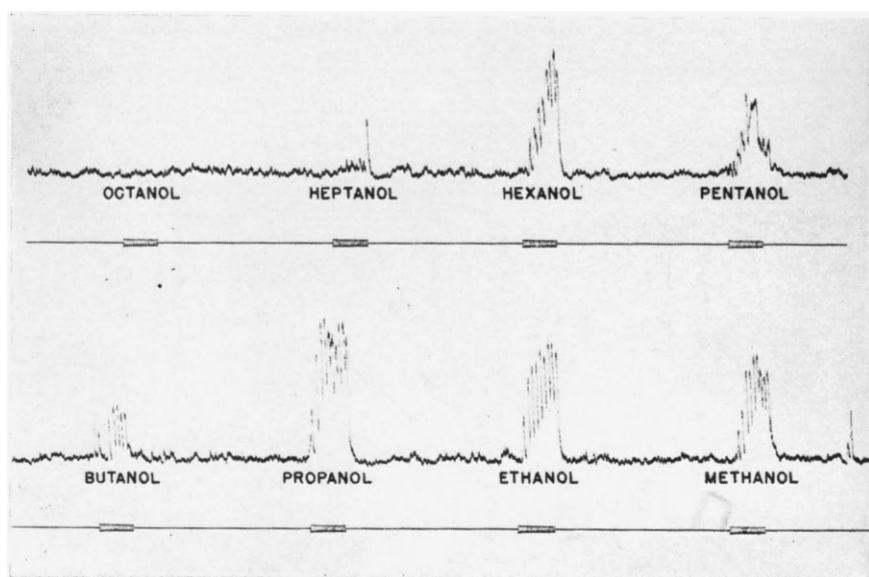
FIG. 20. Integrated trigeminal responses to alcohols. Free respiration and with odor flash held before rabbit's nose for 1 min.

small quantity of liquid odorant. The animal's respiratory rhythm was unusually depressed, which caused exaggeration of the characteristic build-up of the trigeminal response. The trigeminal receptors do not appear to be so exposed to the gaseous environment in the nasal cavity as do the olfactory receptors. However, the trigeminal response can approach its limiting value within two or three inspirations in a preparation respiring briskly. But in such instances the response frequently wanes and waxes repeatedly with continuous application of the stimulus.

The peak in trigeminal response to hexyl alcohol seen in Fig. 20 is typical. In livelier preparations octyl alcohol usually causes a small response. By

contrast, a small olfactory response to decyl alcohol is usually present. Prominent reflex responses of autonomic fibers distributed to the nose occur in parallelism with the trigeminal receptor responses, provided that the animal is not too depressed with urethane anesthesia.   Trigeminal twigs are obtained from the ethmoidal nerve, which arises from the naso-ciliary nerve within the orbit.   At the nasal level the branches of the ethmoidal nerve contain a rich complement of autonomic fibers.   There-fore, one may record trigeminal sensory activity from a peripherally directed twig and autonomic motor activity from a centrally directed twig.

Autonomic Effectors

We know very little about the autonomic effectors and their role in nasal physiology.   Electrical stimulation of the cervical sympathetic nerve commonly causes enhancement of the olfactory response to an odorant (Tucker and Beidler, 1956).   In a preparation suitable for viewing the nasal interior, sympathetic stimulation causes blanching of the nasal mucosa and an increase in width of the slit-like passage between the septum and the ethmoturbinates. The widening undoubtedly increases accessibility of odorant to the olfactory receptors.   Parasympathetic activation by stimu-lation of the greater superficial petrosal nerve seems to cause more profuse flow of nasal secretions.   If the cervical sympathetic nerve is sectioned to prevent sympathetic activity of central origin from appearing at the nasal recording electrodes, presentation of alcohols causes marked increases of parasympathetic activity with trigeminal activation (Fig. 20).

We had long wondered about the basis for synchronization with respira-tion of the automic activity to the nose.   The ongoing level tends to increase with depth of anesthesia and to become more synchronized.   With very deep anesthesia the parasympathetic component is highly depressed. We now know that a mucous rattle in the throat can cause autonomic activity synchronized with respiration, thus is indicated a reflex mechanism.

The ethmoidal nerve site should be of especial interest to students of autonomic nervous system function.   Let us suggest, for example, the application of electrophysiological techniques to the study of drug effects. Small branches of the ethmoidal nerve can easily be dissected free and from them may be recorded action potential spikes of individual fibers belonging to both divisions of the autonomic system.   The wealth of motor innervation is demonstrated by the oscilloscope tracings shown in Fig. 21, although the records are of inferior quality.   Unfortunately, the film driving mechanism in the camera did not maintain constant speed. The responses shown are far from the maxima, rather the stimulus was adjusted for approximately equal postganglionic bursts from the superior cervical sympathetic ganglion and the sphenopalatine ganglion.   The stimulus sites were the cervical sympathetic nerve and the greater super-

ficial petrosal nerve at the point where it arises from the facial nerve. The
sympathetic nerve was sectioned centrally whereas the facial nerve was not.
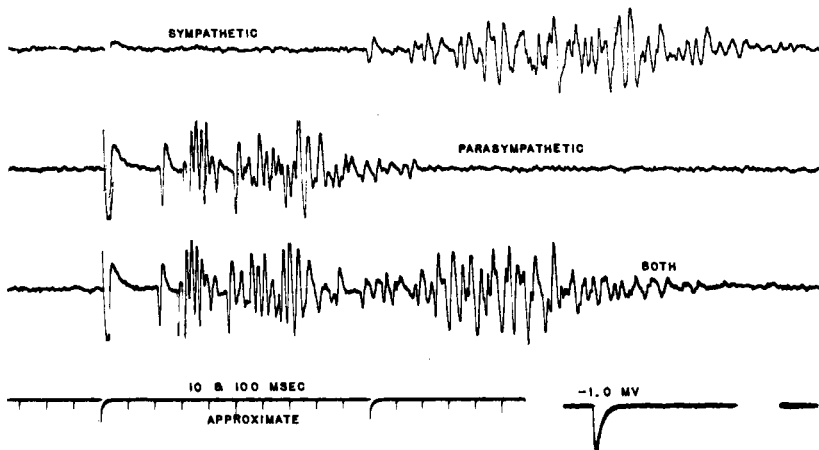
FIG. 21. Postganglionic autonomic responses recorded from ethmoidal nerve
twig for stimulation with 2 msec pulses at preganglionic sites.

CONCLUSION

The kind of odorant and its concentration in the air inspired into the
nose rank as obvious determinants of nasal chemoreceptor responses.
With these must be classified the volume flow rate of odorous medium into
the naris. Either the rate of delivery of odorant molecules to an olfactory
receptor or the effective concentration attained at the receptor within a
characteristic time must be the crucial factor for generating the phasic
response. The latter alternative is consonant with the phasic olfactory
response data for the tortoise. But it is interesting that Stuiver (1958)
chose to consider the stimulus in terms of the rate of absorption of odorant
per unit time and mucosal area. He devised an ingenious equation that
exhibits flow rate dependence and plateauing behavior, but his assumption
that all odorants should be quantitatively similar in these respects appears
inconsistent with the results for amyl acetate and benzyl amine shown in
Fig. 13. Nevertheless, the idea is expressed clearly that odorant is lost
from the flowing medium to the mucous membranes lining the nasal
passages. Therefore, odorant concentration is progressively reduced at
more downstream points.

The assumption that concentration equilibria are quickly established
between the media of the inspired air, the mucus and the receptor sites
(Moncrieff, 1955 ; Davies and Taylor, 1957) appears most applicable to
odorants that are slightly soluble in the mucus. Perhaps one should

reserve the proviso that no other binding mechanism of appreciable capacity be active. Stuiver's (1958) assumption that practically all the odorant molecules striking the mucous surface are trapped indefinitely long seems most applicable to odorants of high aqueous solubility.

With increase in capacity of any trapping mechanism there should, of course, be an increase in flow dependence for the attainment of a given concentration of odorant molecules at some point within the mucus. The kinetics of stimulus transport to the vomeronasal and trigeminal receptors presents interesting problems for study.

ACKNOWLEDGEMENT

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ELECTRICAL ACTIVITY IN THE OLFACTORY SYSTEM OF RABBITS WITH INDWELLING ELECTRODES

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INTRODUCTION

ATTEMPTS to determine how the olfactory bulb deals with the massive input from its receptors, and what its discharges reveal about the way odors are discriminated, have largely been restricted to studies on animals immobilized by surgery or drugs ; or on the wave activity in the bulb of chronically implanted animals (e.g. Adrian, 1950 ; 1956 ; Mozell and Pfaffmann, 1954 ; Mozell, 1958 ; Hernandez-Peon et al., 1960). However, for a fuller understanding of olfactory processes we must know more about the behavior of the spike potentials of the bulb, and their relation to events occurring more peripherally, in animals far removed from the effects of surgical trauma, anesthetics or neuromuscular blocking agents. Not only do such compounds profoundly influence central synaptic transmission, but they may also restrict odorant access to the nasal chemoreceptors by altering the flow of nasal secretions, or the degree of vascular engorgement of the nasal mucosa. A sensory system does not normally function in isolation : its full exploitation demands a complex interplay of activities in accessory structures; and using electrophysiological methods we are likely to learn more about its potentialities and its significance for behavior when it is being actively focused by the animal, than when it is being artificially stimulated by the experimenter.

The approach to these problems through the use of animals with chronically implanted electrodes is particularly favored by the anatomy of the olfactory system. In macrosmatic species, such as the rabbit, the large bulbs and numerous receptor cell axons laced through the cribriform plate allow activity to be recorded simultaneously from both primary and secondary neurones, with little disruption of overlying tissue. In addition, and despite their rich content of autonomic fibers, the nasal mucosa and the ethmoidal nerve are also interesting targets, particularly in view of the potential importance of trigeminal sensitivity in the discrimination of odors (see Tucker, this symposium). We have recently begun to investigate the

influence of odors on the activity which can be recorded from these various sites. In this interim report the nature of this activity and attempts to quantify the spike discharges of the bulb will be described.

METHODS

With sterile precautions, bipolar stainless steel electrodes (127μ and 63.5μ in diameter) were advanced through the floor of a lucite chamber attached to the skull of a young rabbit anesthetized with pentobarbital sodium. When the appropriate activity was identified on the oscilloscope and audio monitor the electrodes were fixed with dental cement and their free ends soldered to connectors housed in the lucite container. Penicillin was administered for four days following the operation.

During trials the rabbit wore a Teflon-coated fiber-glass face mask and was loosely restrained, without compression, in an open wooden box inside a sound-resistant chamber. The chamber had a one-way glass panel through which the behavior of the animal could be observed. Glass and Teflon tubing directed a stream of air, filtered through activated carbon and silica gel, into the face mask. When necessary, this stream could be combined in known proportions with air saturated with a test odorant. Non-olfactory stimuli included shocks delivered across the chest of the animal by way of electrodes stuck to the skin, and clicks (20/sec for 5 sec) delivered into a loudspeaker inside the chamber. Both stimuli were generated by a Grass S4 Stimulator. To provide visual disturbance, the chamber light was turned off for 5 sec in a darkened room. Before trials began, rabbits were placed individually in the experimental situation for a minimum of 2 hr daily until the heart beat declined to a relatively stable plateau (180-200/min). Activity from the electrodes was led through preamplifiers summated on short-term averaging circuits (Beidler's integrator), and displayed on a multichannel pen recorder. Variable electronic band-pass filters were used to filter out the slow wave activity of the bulb when appropriate.

RESULTS

Peripheral Activity

A variety of discharge patterns were recorded from sites in the nasal mucosa but many showed little or no consistent relation to odor presentation. Some appeared when no stimulus was being deliberately applied by the experimenter ; others were especially prominent after an animal had received a shock or novel stimulus to any modality tested. These consisted of prolonged trains of spike discharges up to 200μV in amplitude. At two such sites, there seemed to be a correlation with changes in the rate of heart beat (Fig. 1).

At some peripheral sites, however, responses were more specifically related to odor stimulation, and examples of these—one from non-olfactory
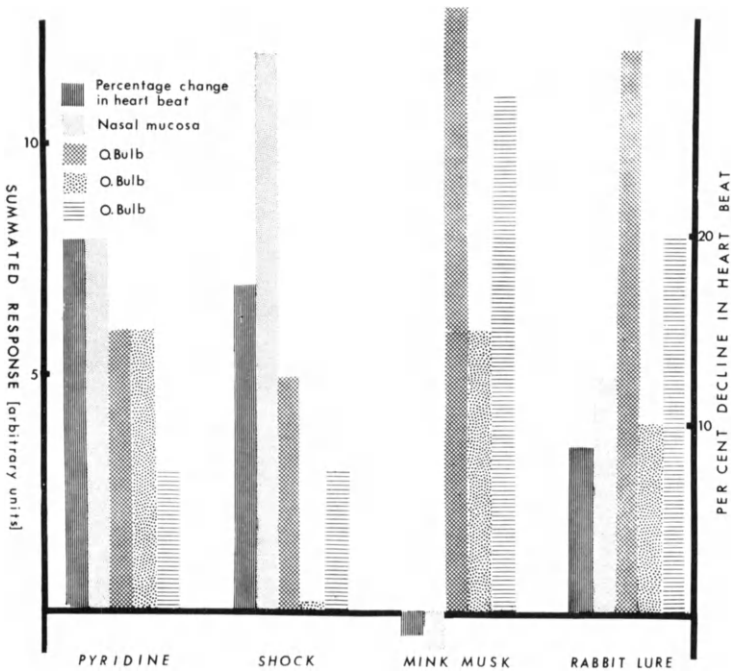
FIG. 1. Bar graph of interrelations between summated discharges recorded from peripheral and 3 bulbar leads, and changes in the rate of heart beat (declines in rate are positive). Although shock and most novel stimuli evoked declines of up to 27 per cent in heart beat and increased activity recorded from certain peripheral sites, mink musk had the reverse effect. Heart beat values relate to a 30 sec period before, and one after stimulus presentation.

filtered air

amyl acetate

50μV

25m.sec
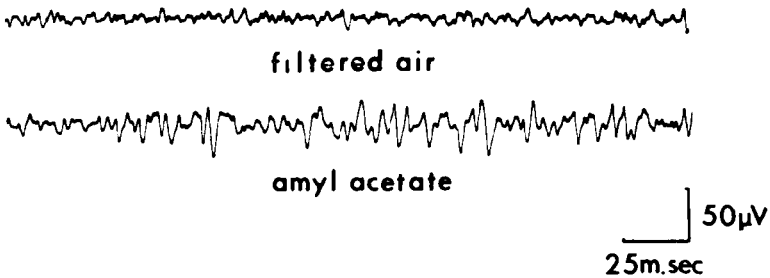
FIG. 2. Pattern of neural discharge recorded from electrodes chronically implanted in a region of non-olfactory nasal mucosa.

mucosa, the other from the region of the cribriform plate—are shown in Figs. 2, 3 and 4. At both sites, the activity consists of asynchronous trains reaching 50μV in amplitude with individual deflections up to 5 msec in

7

duration. The most active responses were generally obtained from
electrodes directed into the cribriform plate from the bulbar side, and,
as can be seen in Fig. 4, they ocurred whenever spike discharges in response
to odor appeared in the bulb. The stimulus–response curves for the two
types of activity cover similar intensity ranges and both responded to
4.39 log M amyl acetate (Figs. 10 and 11).

filtered air

eugenol   10 m.sec   50µV
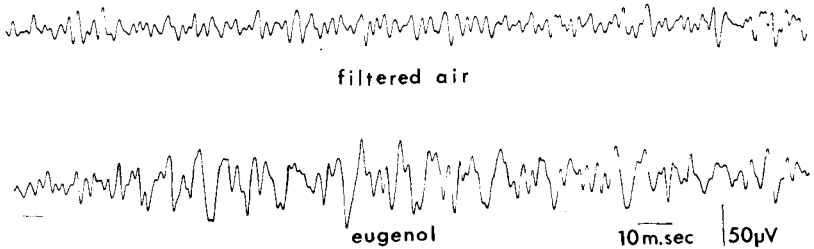
FIG. 3. Activity recorded from electrodes directed into the cribriform plate
through which pass the primary olfactory neurones. When the undisturbed
animal is breathing filtered air there is a higher discharge than that seen in Fig. 2
(top trace)

a   50µV

b   100µV

Filtered   air

a

b

Amyl   acetate

                                                1 sec

FIG. 4. Activity derived from the same site as were the records in Fig. 2 (but at
slower film speed), compared with bulbar spike potentials recorded simultan-
eously. (a) periphery, (b) bulb.

In one animal a spectrum of slow and fast fiber activity was recorded
from electrodes chronically implanted under direct visual control into the
ethmoidal nerve as it enters the cribriform plate. Odor stimulation was
generally followed by a rapid increase of summated activity to a steady
state, more stable, and with a different pattern than that shown by the
bulbar spike discharges. In particular the marked after-discharges which
sometimes appear in the bulb were seldom reflected in the activity of this
nerve (Fig. 7).

Wave Activity of the Bulb

When an undisturbed rabbit was breathing filtered air, low amplitude
asynchronous waves sometimes showing frequencies in the range of

75–90 cps, could be recorded from the bulb. Those gave way to flares of high amplitude almost sinusoidal waves most commonly appearing with each inspiration, when an odor was presented (Fig. 5). In each flare, two or three components can often be distinguished : an initial burst in the range 75–85 cps, the main train of high amplitude waves about 50–70 cps, and a final phase of lower amplitude waves about 40–50 cps. While other frequencies were also recorded from the bulb, (7, 10–14, 20–28 cps), the 50–70 cps rhythm is the most characteristic. Disconformity of wave and spike discharges was frequent—isolated flares appearing when a decline, or little or no change in the level of the spike discharges was apparent. A discrepancy of this kind was also observed when the rabbit was under light nembutal anaesthesia (Fig. 8). Conversely, the period of maximum spike responses to an odorant may coincide with a temporary disappearance of the wave flares. This evidence suggests that the wave and spike activity is generated by different structures.

0·2 mV

0·1 sec
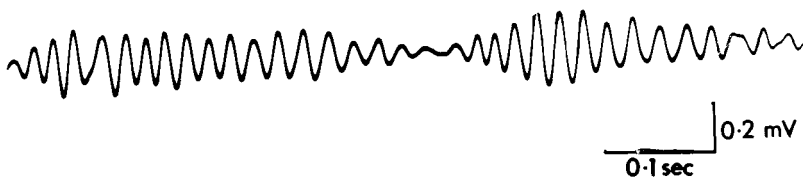
FIG. 5. Two wave flares from the bulb. The main and terminal phase of each flare often show different frequencies. Thus in one animal deer musk evoked a main phase of about 60 cps and a terminal phase of 50–60 cps, while coyote urine elicited a main phase of 70 cps, and a terminal flare of 40–45 cps. An initial phase of 75–85 cps is less often distinguishable.

Bulbar Spike Potentials

A continuous irregular discharge of spikes is normally present in the bulb when an undisturbed rabbit is breathing filtered air. What happens when an odor is introduced depends, at least partly, on the type and concentration of the odor, on the frequency with which the animal has been previously exposed to it, and on placement—a common pattern being that shown in Fig. 6. Here the spikes become grouped with each inspiration, but in the interburst intervals there is a pause in the rate of firing, which may carry activity to a level below that of the resting discharge.

Such a pattern seldom summates effectively against the fluctuating baseline, even although counter records may indicate a marked increase in the frequency of high amplitude spikes. Novel odorants (i.e. odorants which had not previously been presented to the animal) evoked the most prominent spike discharges (Fig. 7), but their effectiveness generally declined with repetition and no consistent relation between the amplitude of the summated response and the baseline for any concentrations of the odorant tested could be established. The time of appearance of the response

Filtered air

Oil of Spearmint

Pyridine

Experimenter visible

0·5mV

0·5 sec

FIG. 6. Bulbar spike potentials. The odorants were introduced at about the point where the records begin. In the upper trace the animal is undisturbed and apparently relaxed. In the bottom trace the animal is breathing filtered air, the chamber light has been turned off, and the experimenter is visible to the rabbit. Note the fluctuating phases of activation and deactivation in the spike discharges of this last trace.

a

b

c

d

OIL OF SPEARMINT

CIGARETTE SMOKE

a

b

c

d

MINK MUSK

RABBIT LURE

a, b, c: olfactory bulb   d: ethmoidal nerve   5 sec
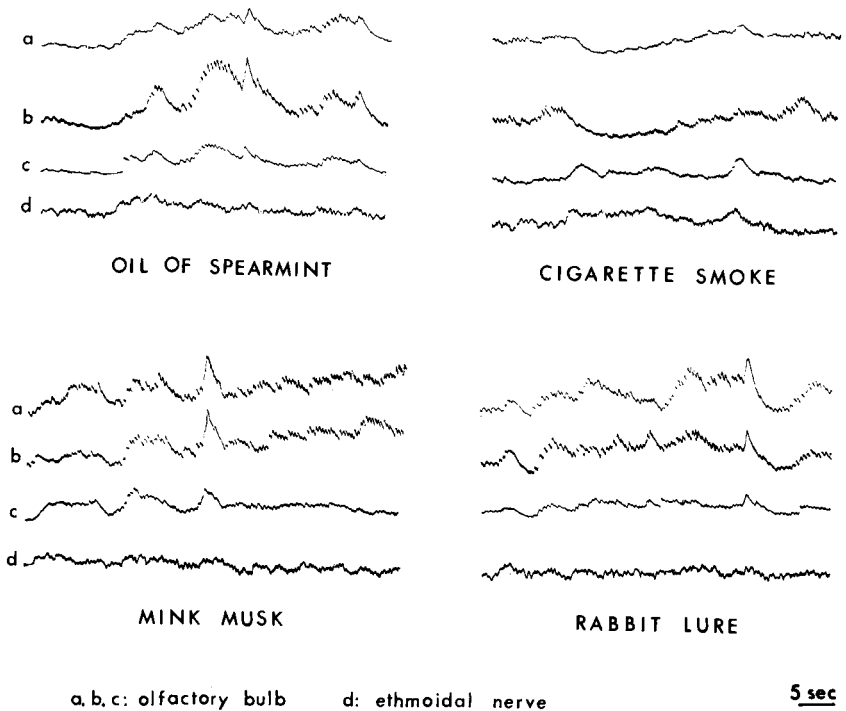
FIG. 7. Summated responses to odor recorded from three sites in the bulb and one site in the ethmoidal nerve.

also varied, and when an odorant in medium or relatively high concentrations evoked little activity during its presentation, a strong discharge frequently followed several seconds later.   At lower concentrations, however, responses more often occurred within the duration of the stimulus. In spite of these discrepancies, qualitative differences in the pattern of summated response evoked by different odorants are apparent, and there are marked variations in the discharges recorded simultaneously from different points in the bulb (Fig. 7).

Several attempts to stabilize the response were made.   Of these, preliminary results showed that pairing unavoidable shock with an odorant to which the animal was habituated, resulted in an enhancement of wave activity within the first 23 trials, but for a fixed stimulus, the relation of the summated spike response to baseline remained inconsistent.   A further approach concerns the efferent fiber system.

Spike Activity in the Bulb Following Unilateral Frontal Transection of Anterior Olfactory Areas

There is now extensive evidence that efferent fiber systems are at least potentially capable of mediating central control of activity in the bulb; and, indeed, it is difficult to see how the massive projection recently described by Cragg can have no important function in olfaction.   In addition to fibers originating in rhinencephalic or higher centers and terminating in some cases as far down as the perimeter of the glomeruli, there are also fine fibers interconnecting the bulbs.   Although these may be too few to have much functional significance, some interaction of bulbar activity, possibly mediated in part by other pathways, may normally occur.   Thus Kerr has shown that strong olfactory stimulation of one bulb can suppress afferent induced waves in the opposite bulb (Cajal, 1911 ; Adey, 1953 ; Cragg, 1962 ; Kerr and Hagbarth, 1955 ; Walsh, 1959 ; Kerr, 1960 ; Hernandez-Peon *et al*, 1960 ; Yamamoto and Iwama, 1961 ; von Baumgarten *et al*, 1962).   However, the extra-bulbar efferent pathways, particularly the autonomic supply to the nasal mucosa (Tucker and Beidler, 1956), are capable of mediating powerful influences on olfactory sensitivity which may account for some findings in this area.

On the assumption that much of the variability of the bulb responses in the unanaesthetized rabbit was related to the activity of bulbar efferent fibers, the effect of unilaterally transecting anterior olfactory areas (including the anterior commissure and prepyriform cortex) 5–9 mm posterior to the caudal end of the olfactory bulb was investigated.   The intention was to sever all efferent fibers to one bulb and the interbulbar fibers to the other. Cragg (1962) could find little evidence of retrograde degeneration in the bulb following lesions in this area.

As Fig. 8 suggests, the effects of such a lesion were striking.   On the side

of the transection, spike discharges in response to odor stimulation stand
out clearly against the low amplitude background activity and they sum-
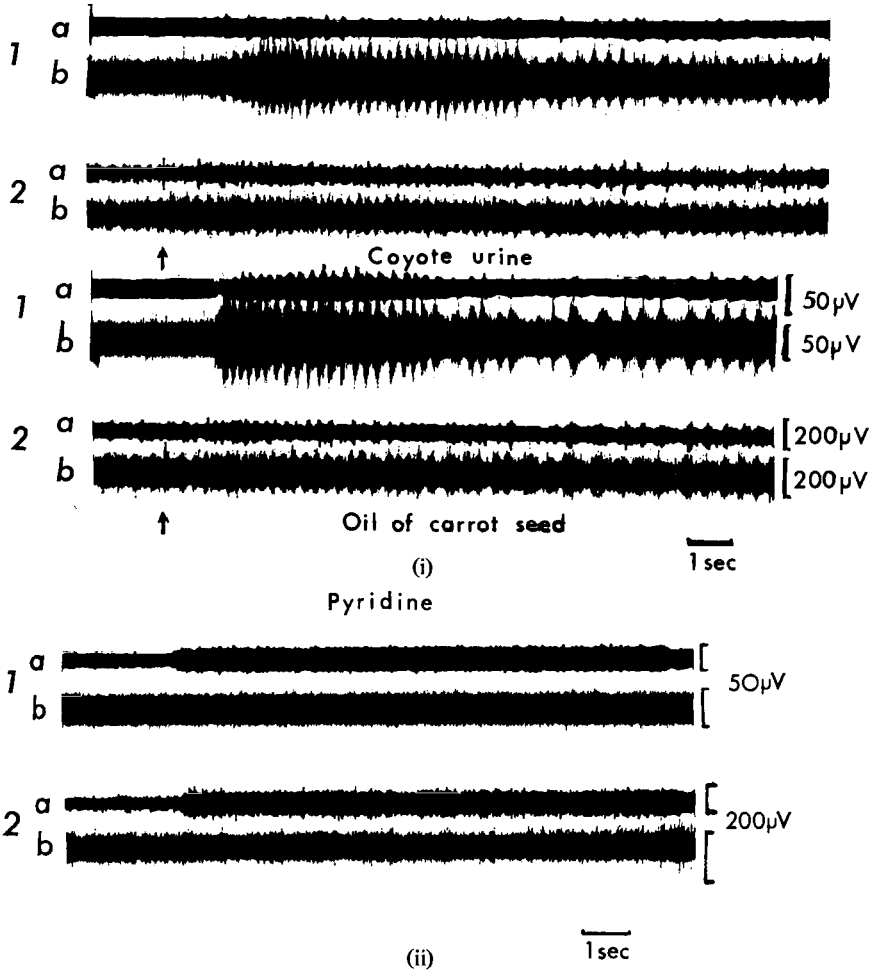
FIG. 8 (i) and (ii). Waves (a) and spike (b) discharges in response to odorants in
the bulbs of a rabbit with unilaterally transected anterior olfactory areas. Each
set of four traces (1 and 2) was recorded simultaneously. 1, Side of lesion.
2, Opposite side. In (i) the animal was unanaesthetized and the odorants were
introduced at about the point indicated by the arrow. In (ii) the animal was
under light nembutal anaesthesia and the odorant was introduced about 50 sec
previously for 10 sec, but elicited no immediate response. Note that the train of
waves is unaccompanied by any marked change in spike activity in either bulb.
Within a bulb the same pair of electrodes was used to record both the spikes and
the waves, the activity being led through 2 band pass filters at different settings.

mated strongly. On the opposite side, responses are largely embedded in
the high amplitude resting discharge, as in the rabbits with intact efferent

fibers, and they summated poorly : however, from some sites effective integrator records were obtained although they were generally less promi- nent than the responses on the side of the lesion.   In such a preparation, repeated presentation of the same odorant failed to produce any diminution (i.e. habituation) of response.   Furthermore, as Fig. 9 shows, the responses tend to rise with increasing concentrations of amyl acetate.   This effect
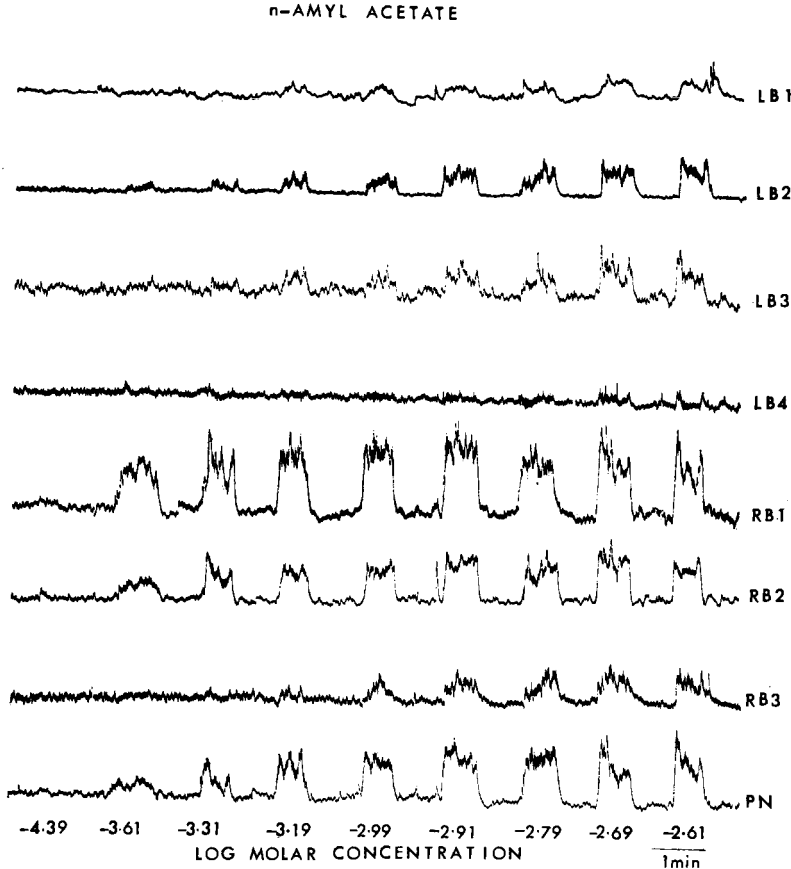
n–AMYL ACETATE

FIG. 9. Summated responses to an amyl acetate concentration series. Spike dis- charges from bulbar sites are compared with activity recorded simultaneously from electrodes implanted in the cribriform plate region (P.N.). The recorder was turned off for a minimum of 5 min between successive presentations of a stimulus.

is seen more clearly in Fig. 10, where stimulus-response curves derived from 7 bulbar sites and one peripheral site are compared.   With one exception the curves of lowest amplitude were derived from bulbar points on the side opposite the lesion.   The peripheral curve (P.N.), and several bulbar curves show little tendency to flatten at the highest concentrations

tested although at some sites there are marked reversals or declines at
this level. Also one lead yielded two curves. But in spite of these pecu-
liarities the overall trend is apparent, and it is clear that the peripheral
activity is closely related to the bulbar discharges.
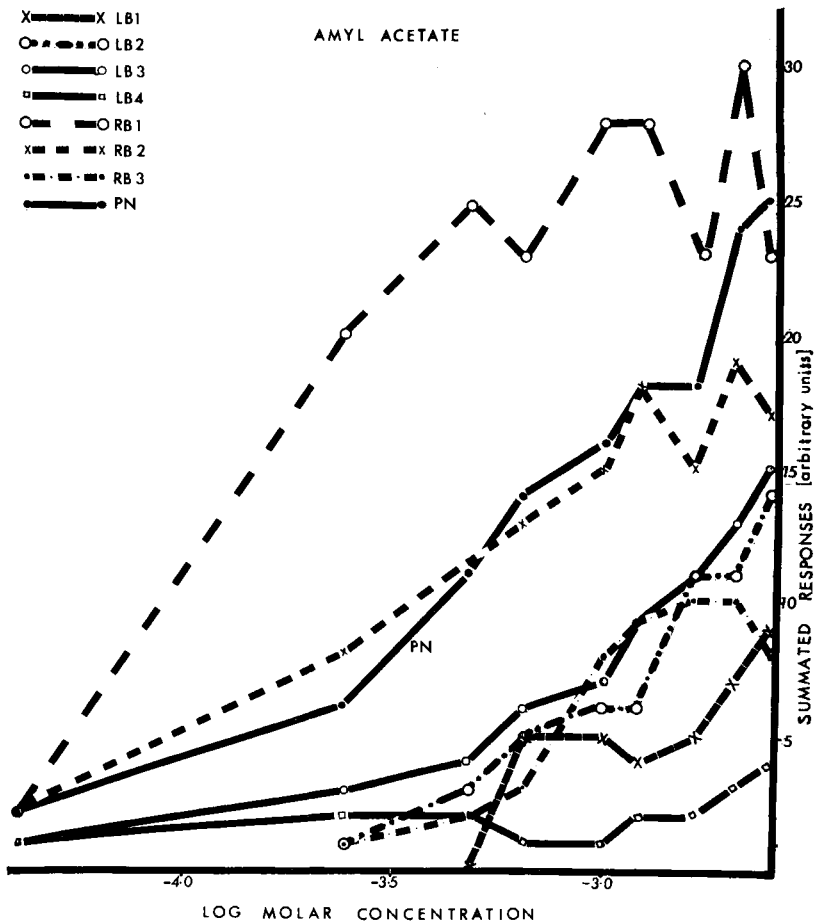
FIG. 10. Stimulus–response curves for amyl acetate derived from a rabbit with
a unilateral transection of anterior olfactory areas caudal to the bulb. Sum-
mated spike discharges RB 1–3 from the bulb on the side of the lesion ; LB 1–4
from the bulb on the opposite side, and P.N. from the cribriform plate region.
Numbering begins at the back of the bulb.

Simultaneous recordings from the 8 points also reveal marked contrasts
in the integrated responses to various odors at different sites in the system.
Thus, Fig. 11 shows that 2-butanone was an effective stimulus at RB 1,
while eugenol had little or no effect. Conversely, activity at LB 2 was
hardly influenced by 2-butanone, although eugenol elicited a significant

discharge.   In the case of heptane, there is a secondary peak whose height relative to the initial peak varies for different placements.

BENZENE   HEPTANE   2-BUTANONE   AMYL   EUGENOL
ALCOHOL
CONTROL (alternate responses): AMYL ACETATE   1 min.
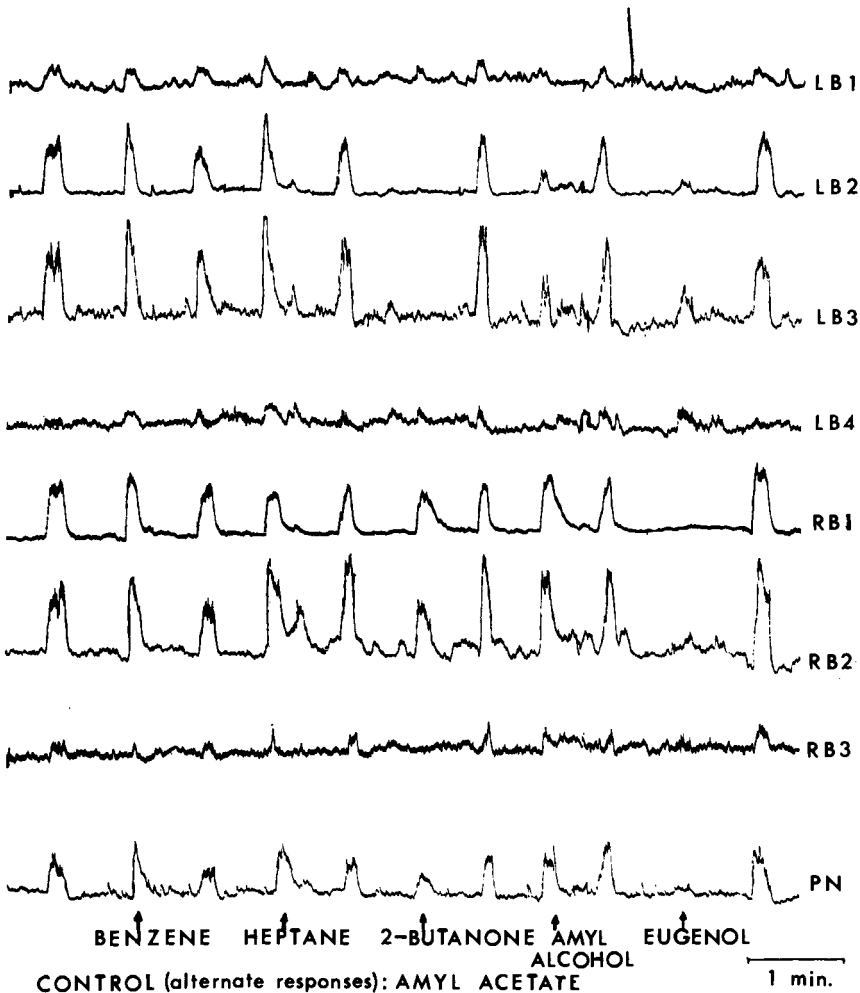
FIG. 11.   Integrator records for responses to different odorants in a rabbit with a unilateral transection of the anterior olfactory areas.   Symbols are as in Fig. 9. The same concentration of amyl acetate was presented before and after each test of a different odorant (intervals of at least 5 min between successive stimuli).

A further point illustrated by Fig. 11 relates to the control stimulus, amyl acetate. Although there is a relatively high consistency in the amplitude of the responses, especially when averages are compared, it is clear that the relative effectiveness of this odorant at different sites is not always constant.

DISCUSSION
Peripheral Activity

The rich trigeminal and autonomic innervation of the nasal mucosa makes it probable that the activity recorded from the non-olfactory regions was largely derived from such fibers. However, since sites especially responsive to odorants were located, some isolation of trigeminal activity may be possible. In the case of recordings from electrodes directed into the cribriform plate, a different source for the potentials seems likely. In this area the primary olfactory neurones form numerous bundles coursing towards the bulb and they are easily accessible to electrodes inserted from the bulbar side. The close correlation between the amyl acetate stimulus–response curves for the bulbar and peripheral sites (Fig. 10) and the nature of these peripheral discharges (Figs. 3 and 4), indicates that contact with such bundles was probably made. It is also significant that this site yielded responses to amyl acetate in concentrations at least 1 log unit below the limit for trigeminal sensitivity to this compound which Tucker (this symposium) has determined for the anaesthetized rabbit. Indeed thresholds for bulbar spike discharges and the peripheral activity are virtually identical (Fig. 10).

Bulbar Activity

The behaviour of the spike discharges in the lightly or recently anaesthetized rabbit has been described by Adrian (1950), and Mozell and Pfaffmann (1954). It appears to differ from that found in the present study mainly in the degree of stability of the background discharge. In the most extreme situation, such as is seen when the animal has been shocked, or the experimenter is visible (Fig. 5), bursts of activity may alternate with periods of marked deactivation, in which few individual spikes appear above a constricted baseline. These are quite distinct from the changes occurring with each inspiration. It is tempting to speculate that this may be related to rapid transfers of attention from one modality to another. Even in the undisturbed animal apparently " spontaneous " shifts in the intensity of this activity occur which maintain the baseline, sometimes for prolonged periods, at higher or lower levels. The introduction of an odorant at any suprathreshold concentration may initially supress this activity—with or without eliciting spikes at each inspiration—or induced spikes may appear to be superimposed on the resting discharge. This contrasts with the peripheral discharges of the kind shown in Fig. 4, in which the response to an odorant is generally an increase in activity above the baseline, which remains relatively stable.

The effect of lesions in the anterior olfactory areas is apparently to shift the behaviour of the bulbar spike discharges to a condition more closely approaching that seen at the periphery. Indeed, some bulbar sites

show responses to odor, which, when summated, are virtually indistin-
guishable from peripheral activity. In this condition relatively consistent
responses to odor occur and a quantitative analysis of both peripheral
and bulbar spike discharges becomes possible.

Before considering the possible causes of this effect it is convenient to
distinguish two levels of resting discharge : a primary level of low ampli-
tude stable activity seen in deeply anaesthetized animals, and a secondary
level of higher amplitude irregular spike discharges which appear in
unanaesthetized or lightly anaesthetized animals. Examination of Fig. 8
suggests that the secondary level of resting discharge is absent or reduced
on the side of the lesion, leaving mainly a low amplitude, stable activity
comparable to the primary resting discharge.   On the opposite side,
however, the fluctuating secondary activity still appears to be present,
although some reduction may have occurred. Thus, although the spikes
elicited by odor are of similar amplitude in both bulbs, those on the side
of the lesion stand out more clearly than those on the opposite side, which
are embedded in the secondary resting discharge.

Such evidence suggests that activity in the efferent fiber systems may
normally activate the secondary resting discharge, possibly adjusting it to
a level appropriate to the biological significance of the afferent inflow to
the bulb. However, the possibility that some enhancement of the induced
spikes may also occur cannot be ruled out on the basis of available evidence.

Similarly there is insufficient evidence to allow any generalizations about
the relative effectiveness of different odorants in stimulating various points
in the bulb or the periphery in this preparation, but the existence of
temporal and spatial patterning of responses to different odors, previously
demonstrated by Adrian (1956) and Mozell and Pfaffman (1954), is
apparent.

SUMMARY

The electrical responses of the olfactory bulbs and peripheral olfactory
system to odor stimulation were explored in rabbits with chronically
implanted electrodes.   There were marked disconformities between the
behavior of the spike and wave activity of the bulb, and for a given stimulus
the spike response did not summate effectively or consistently against the
fluctuating high amplitude resting discharge present in the unanaesthetized
animal.   The variability was greatly reduced by a unilateral lesion in
the anterior olfactory areas through which pass the efferent fibers to the
bulb.   In such a preparation bulbar spike responses on the side of the
lesion especially stood out against a stable low amplitude resting discharge,
and they summated effectively and consistently.   Repeated presentations
of the same olfactory stimulus led to no diminution (habituation) of

response. The bulbar spike discharges correlated closely with activity assumed to be that of the primary olfactory neurones and recorded with electrodes implanted in the region of the cribriform plate. For this preparation stimulus–response curves derived from simultaneous recordings of summated spike responses to amyl acetate at eight points in the bulb and periphery, are presented. Differences between odorants in their relative efficiency in stimulating different points in the bulbs and at the periphery are also described.

ACKNOWLEDGEMENTS

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ELECTROPHYSIOLOGICAL INVESTIGATION OF INSECT OLFACTION

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THE SENSE ORGAN

INSECTS possess well-developed chemical senses. But unlike the verte-
brates, their sense organs for these chemical modalities are located at the
body surface uncovered by mucous and readily accessible to the surround-
ing medium. During development of the insect cuticle two daughter epi-
dermal cells differentiate to govern the formation of a specialized area of
the body surface which will serve as a contact locus for certain sensory
nerve fibres. One of these two cells—the trichogenic cell—builds a hair-,
peg-, cup- or plate-like cuticular piece. The other—the tormogenic cell—
builds a ring or socket surrounding this area. Sensory nerve cells such as
the formative cells of the specialized part of cuticle are also derived from
epidermal cells by differential cell division. This developmental plan is
similar for all sense organs of the insect cuticle as well as for non-sensory
hairs, scales, and glands (Henke, 1953). Each of these morphologically
differentiated pieces of insect cuticle with its formative cells, sensory-nerve
cells and accessory cells is called a *Sensillum* (Fig. 1).

To each sensillum belong from one to many nerve cells. The distal
process of the nerve fibre grows " through " the trichogenic cell body to
make contact with the cuticle. In unequivocally identified gustatory
chemoreceptors, the endings of each nerve cell are in direct contact with
the surrounding medium through an opening at the tip of the hair (Dethier,
1955). Peg-shaped sensilla basiconica, probably serving the olfactory
modality, have many cuticular pores where the dentritic processes of the
nerve cell are in contact with the outer medium (Slifer, Prestage and Beams,
1957).

A single sensillum does not necessarily serve only one sensory modality,
as was shown in the fly. Here a single hair is supplied by the fibres of
several nerve cells, one of which ends at the hair base in the socket area
and responds to mechanical deflections of the lever-like hair. The others—
usually two—are chemoreceptive. One responds to " acceptable " sub-
stances such as sugar ; the other responds to "unacceptable" substances

such as salt (Dethier, 1955 ; Hodgson, Lettvin and Roeder, 1955 ; Wol-
barsht and Dethier, 1958).

In connection with investigations of insect chemo-reception two ques-
tions have been repeatedly raised:

1. *Is it possible to distinguish between the " olfactory " and " gustatory "*
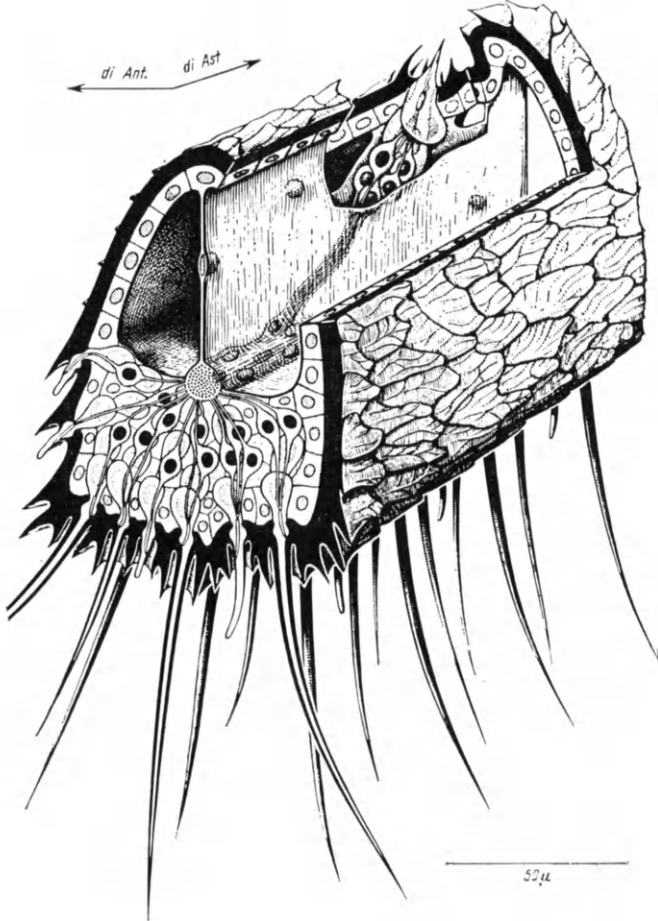
FIG. 1. Three-dimensional representation of a portion of a *Bombyx* antennal
branch showing short, thin-walled sensilla basiconica and long, thick-walled
sensilla trichodea (below) and one sensillum coeloconicum (above) (from
Schneider and Kaissling, 1959).

modalities, as in the case of mammals?—There are receptors which respond
specifically to water-soluble substances such as salt and sugar. Other
receptors are specifically responsive to air- or water-borne substances
which are water-insoluble or water- and lipoid-soluble, respectively. Both
receptors may be found side by side, not necessarily localized in restricted

areas or organs as in vertebrates (cf. also Dethier and Chadwick, 1948).

2. *Which type of sensillum is responsible for olfactory reactions?*—The answer to this question was gradually approached by earlier observations which localized the main group of olfactory receptors on the antenna (v. Frisch, 1919). But only in a few exceptionally fortunate cases was it possible to identify the olfactory receptor proper. Notable examples, accomplished by coating or amputation of sensilla, are the experiments of Dethier (1941) and Bolwig (1946) with larvae of lepidoptera and diptera, respectively, and of Wigglesworth (1941) with the human louse. In these cases the sensilla basiconica were found sufficiently separated to be made individually inoperative. The results showed that olfactory reactions of the animals were not elicited after selective elimination of these sensilla. Most of the other reported cases are still uncertain because of the presence of many different types of sensilla packed closely together. Here, no proof for an olfactory function of a specific sensillum could be obtained with classical methods. Very recently, however, Morita and Yamashita (1961) using improved electrophysiological techniques on *Bombyx* larvae as well as Boeckh (1962) and Schneider and Boeckh (1962) working with beetles and moths, presented direct evidence for the olfactory function of sensilla basiconica (Fig. 2).

ELECTROPHYSIOLOGICAL STUDIES OF
INSECT OLFACTORY RECEPTORS

The first successful investigations of this kind recorded multi-unit bursts of nerve impulses from the antennae of cockroaches, bees and flies (Boistel, 1953, 1960 ; Roys, 1954 ; Smyth and Roys, 1955 ; Boistel, Lecompte and Coraboeuf, 1956 ; Hodgson, 1958). The unknown receptors responded to comparatively strong olfactory stimuli with increased impulse frequencies.

In our laboratory we also tried to record the responses of highly specialized olfactory receptors serving in the sexual behaviour of the silkworm and other moths. In these cases it was not only possible to record the frequency modulation of olfactory nerve impulses but also a *slow olfactory potential* (Fig. 3) called the *electroantennogram* or EAG (Schneider, 1955; Schneider and Hecker, 1956; Schneider, 1957a and b ; Schneider, 1962).

We suggested that the EAG is essentially the sum of many olfactory receptor potentials recorded more or less simultaneously by an electrode located in the sensory epithelium. This assumption has now received experimental support, since we know that the single unit receptor potential appears identical (cf. Fig. 2 with Fig. 3B and C). With extracellular recording, the unit receptor potential and the EAG usually show the expected polarity. The receptor site temporarily becomes negative, in relation to the reference point.

FOOD DETECTION BY OLFACTORY RECEPTORS

As is well known, the olfactory sense serves insects in different ways. Many insects depend largely upon their olfactory receptors to find food or places to lay eggs. A good example is the worker honeybee which is
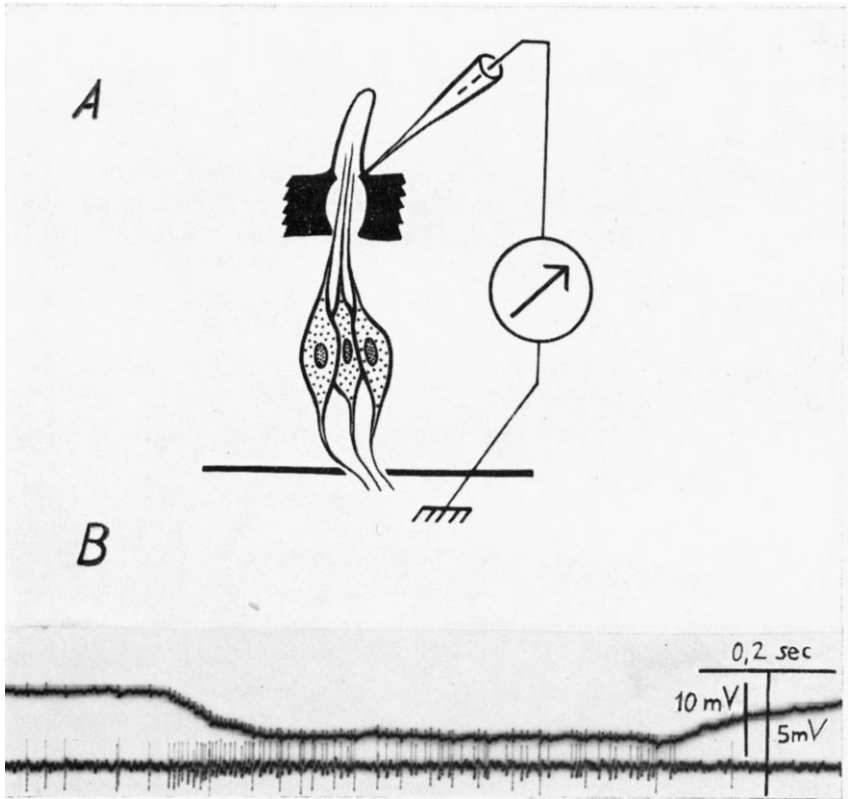
Fig. 2. A, Method of recording action potentials with glass capillary electrodes from a single sensillum basiconicum. B, Recording from *Sphinx pinastri* (Sphingidae) stimulated with terpineol. Three units are active. Upper (D–C) trace shows receptor potential with nerve impulses ; lower (R–C) trace shows only nerve impulses. Downward deflection indicates negative charging of the recording electrode (from Schneider and Lacher, unpublished).

directed to a large extent by olfactory stimuli during foraging. Rather unexpectedly the bee was shown to have a sense of smell somewhat similar to man (v. Frisch, 1921). This broadly developed sense does not show extreme sensitivity but enables the animal to distinguish between a large spectrum of odours.

Many *food specialists* among the insects also exist. A well known example is the carrion-beetle of the genus *Necrophorus* (cf. Dethier, 1947).

Boeckh (1962) recently analysed some olfactory receptors of this animal in detail. Among the numerous antennal sensilla of different types and unknown function, he was able to find a uniform field of sensilla basiconica which gave responses to olfactory stimuli. If an electrode is placed at the
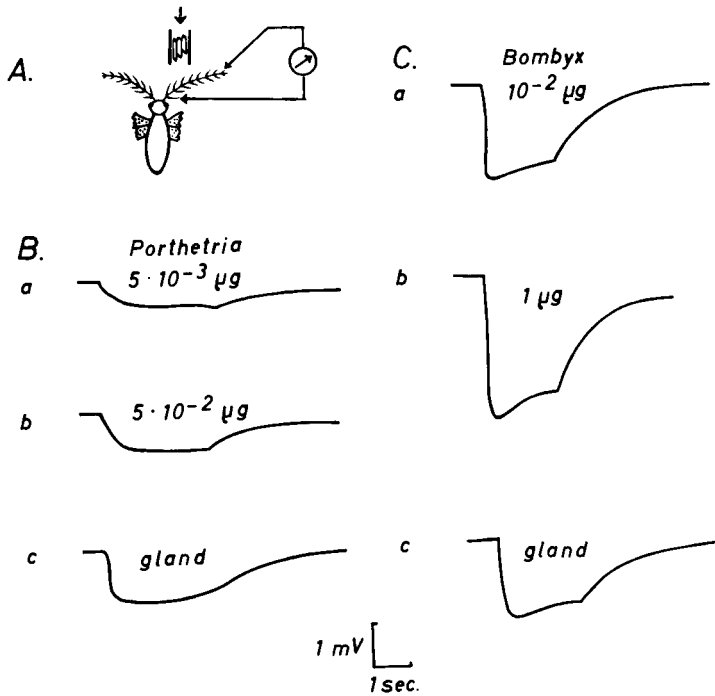
FIG. 3. A, Method of recording electroantennograms (EAG's) from moths. Air is directed toward the antenna through a cartridge containing filter paper impregnated with an odorous solution of known concentration. B, EAG's from *Porthetria dispar*. a and b, Responses to different concentrations of the natural sex attractant. c, Response to the excised gland of a virgin female. C, EAG's from *Bombyx mori*. a and b, Responses to different concentrations of the synthetic sex attractant. c, Response to the excised gland of a virgin female (from Block, Boeckh and Schneider, unpublished).

base of one of these sensilla, apparently supplied by only one sensory nerve fibre, receptor potential and nerve impulses are simultaneously recorded during stimulation. Adequate stimuli are odour currents of decomposing meat. But some chemically defined substances such as mercaptan, fatty acids and amines elicited responses as well. Ammonia and H_2S are without effect upon the sensilla basiconica of *Necrophorus* thus far studied.

The single *Necrophorus* receptor unit either reacts with a depolarization of the receptor site and generation of impulses to stimuli such as rotting meat, mercaptan, amines and homologous fatty acids with between 6 and 10 C-atoms, or with a hyperpolarization of the receptor site and inhibition

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of impulses to stimuli such as cycloheptanon and fatty acids with 3 and 4 C-atoms (Fig. 4).
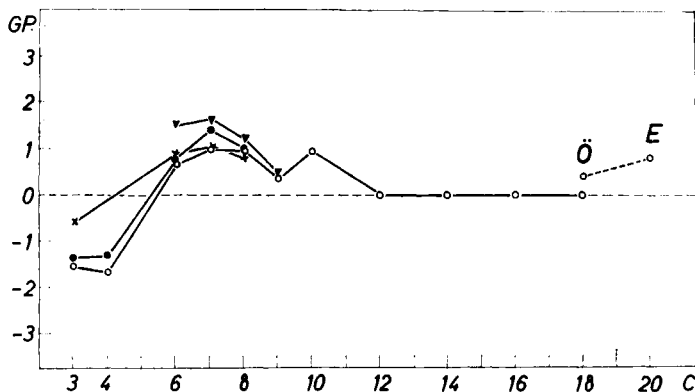
FIG. 4. Relationship of receptor potential (GP) amplitude to chain length (C) of unbranched fatty acids in *Necrophorus humator*. Points on negative side of ordinate represent different degrees of hyperpolarization (from Boeckh, 1962).

RECEPTORS FOR ODOROUS SEX ATTRACTANTS

Odour stimuli not only lead many insects to food or prey but serve as signals for sexual reactions. Best known in this respect are the sexual attracting substances of the silkworm moth *Bombyx mori* and the gypsy moth *Porthetria dispar*. The lure substances of these species, as well as those of other moths, are produced by glands located in the intersegmental folds near the abdominal tip of the female. Intensive work by two groups of biochemists has clarified the chemical nature of the lure substances.

In *Bombyx* the substance has been chemically identified as hexadecadien-10-*trans*, 12-*cis*-ol-(1) (Table 1A) and is called Bombykol (Butenandt, Beckmann, Stamm and Hecker, 1959 ; Hecker, 1960 ; Butenandt, Hecker, Hopp and Koch, 1961 ; Butenandt, Beckmann and Hecker, 1961 ; Butenandt and Hecker, 1961) ; in the gypsy moth the substance has been chemically identified (Table 1B) as [+] 10-acetoxy-*cis*-7-hexadecen-1-ol (Jacobson, Beroza and Jones, 1960 ; Jacobson, 1960 ; Jacobson, Beroza and Jones, 1961).

Behavioural bioassays for the identification of Bombykol (cf. Butenandt, 1955 ; Hecker, 1960 ; Butenandt and Hecker, 1961) demonstrated a very low reaction threshold of *Bombyx* males to the species lure substance. If a glass rod is dipped into solvent containing only $10^{-12}/\mu g/ml$ of attractant and held in front of the males, at least 50 per cent react with typical movements. The other three geometrical isomers of the alcohol are much less effective (Table 1). Typical for the *Bombyx* reaction is the fact that practically all male moths respond within a few decadic concentration steps

TABLE 1.   THRESHOLD CONCENTRATIONS OF THE SEXUAL ATTRACTING SUBSTANCES OF *Bombyx mori* AND *Porthetria dispar*

Form of attracting substance	Behavioural thresholds	Electrophysiological thresholds
A. *Bombyx mori*—geometrical isomers H H H (a) $CH_3-(CH_2)_2-C=C-C=C-(CH_2)_8-CH_2OH$ H Hexadecadien-10-*trans*, 12-*cis*-1-o1	$10^{-12} \mu g/ml^{c, d}$ $10^{-13} \mu g^b$	$10^{-10} \mu g^b$ $10^{-8} \mu g/ml^g$
H H H (b) $CH_3-(CH_2)_2-C=C-C=C-(CH_2)_8-CH_2OH$ H Hexadecadien-10-*cis*, 12-*trans*-1-ol	$10^{-3} \mu g/ml^{c, d}$	$10^{-5} \mu g^b$
H H H H (c) $CH_3-(CH_2)_2-C=C-C=C-(CH_2)_8-CH_2OH$ Hexadecadien-10-*cis*, 12-*cis*-1-ol	$1 \mu g/ml^{c, d}$	$10^{-6} \mu g^b$
H H (d) $CH_3-(CH_2)_2-C=C-C=C-(CH_2)_8-CH_2OH$ H H Hexadecadien-10-*trans*, 12-*trans*-1-ol	$1 \mu g/ml^{c, d}$	$10^{-5} \mu g^b$
B. *Porthetria dispar*—optical isomers H H H (a) $CH_3(CH_2)_5-C-CH_2C=C(CH_2)_4-CH_2OH$ $\mid$ $O-C-CH_3$ $\parallel$ O (+)-10-acetoxy-*cis*-7-hexadecen-1-ol	$10^{-5} \mu g^a$ $10^{-7} \mu g^e$	$10^{-2} \mu g^b$
O $\parallel$ (b) $O-C-CH_3$ $\mid$ H H $CH_3(CH_2)_5-C-CH_2-C=C(CH_2)_4-CH_2OH$ H (−)-10-acetoxy-*cis*-7-hexadecen-1-ol		$10^{-2} \mu g^b$
(c) Racemic Mixture of B, a and b	$10^{-3} \mu g^a$ $10^{-7} \mu g^e$	$10^{-2} \mu g^b$
(d) " Minor " attractant (not yet identified)	$10^{-2} \mu g^f$ $10^{-3} \mu g^a$	

[a] Block (unpublished).
[b] Block, Boeckh and Schneider (unpublished).
[c] Butenandt, Hecker, Hoppe and Koch, 1961.
[d] Hecker, 1960.
[e] Jacobson, Beroza and Jones, 1960.
[f] Jacobson, Beroza and Jones, 1961.
[g] Schneider, 1962.

above a concentration of $10^{-12}/\mu g/ml$ solvent. The saturation level of the motor response, but not necessarily of all the receptors involved, is reached.

Similar work was done on the gypsy moth by B. C. Block (1960 and un-published). This investigator used a different method, mounting a filter paper piece contaminated with known amounts of the *Porthetria* attractant in front of a rack with 30 male moths. With an air current directed toward the rack, the males reacted typically to lure concentrations of above threshold.

The results for *Bombyx* and *Porthetria*, although worked out with dif-ferent methods, have a similar stimulus–response course in common. Beginning from a certain scent concentration, the reaction curve rises as a straight line when the response is plotted linearly and the lure concentration logarithmically (Fig. 5). The upper " saturation " plateau is reached after increasing the stimulus intensity between 100 and 1000 times above
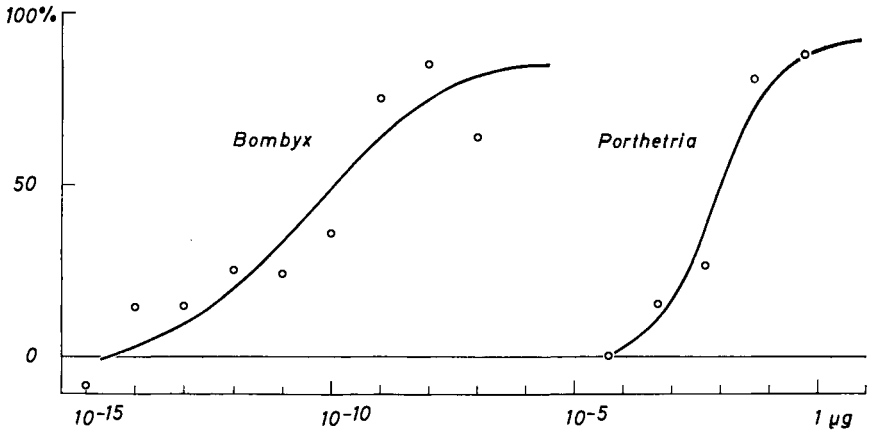
FIG. 5. The behavioural stimulus–response curve of *Porthetria dispar* males to the sexual odour of its own female and of *Bombyx mori* males to the synthetic Bombykol. Ordinate : per cent of males reacting. Abscissa : Amount of lure substance on a stimulus source (from Block, Boeckh and Schneider, un-published).

threshold. The most remarkable difference between these two behavioral investigations is the position of the absolute threshold. Block found that gypsy moth males do not react below $10^{-5}/\mu g$ of substance on filter paper. This differs from *Bombyx*, where less than $10^{-12}/\mu g$ of Bombykol was on the glass rod tip at threshold and cannot be explained by methodology. We must envisage a difference in the absolute reaction threshold of the two species.

In an earlier electrophysiological study, the stimulus–response function of the whole antenna of *Bombyx* males was found to increase as the logarithm of the stimulus concentration (Schneider, 1962). This work

was preliminary, using the amputated antenna and stimulation with synthetic Bombykol on a glass rod as in the initial experiments (Schneider and Hecker, 1956 ; Schneider, 1957). We are now repeating this work with refined methods. The living animal is mounted so that recording is possible with circulatory and respiratory systems intact. Under these circumstances it is possible to record EAG's for many hours or days (Block, Boeckh and Schneider, unpublished). We also changed the stimulating method by using fluted filter paper impregnated with known amounts of lure substance. The filter paper pieces are put in short glass tubes mounted in front of the air outlet (Fig. 3A). Such cartridges can easily be exchanged during the course of an experiment.
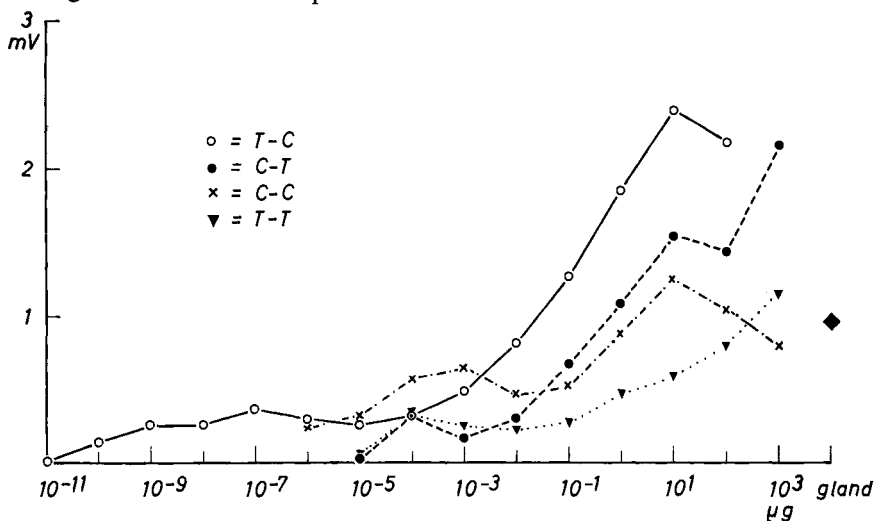
FIG. 6. The relationship between *Bombyx* male EAG amplitudes and concentrations of the four geometrical isomers of hexadekadienol (from Block, Boeckh and Schneider, unpublished).

The results demonstrate that the earlier curve (cf. Fig. 7 in Schneider, 1962, with Fig. 6) is only one of several response types obtained with the *Bombyx* antenna. To compare the earlier curve with the new ones done with the refined stimulation method, the following consideration is necessary. Only about 1/100 of the amount of substance scaled on the abscissa in Fig. 7 of Schneider (1962) actually sticks to the tip surface of the glass rods. Therefore $10^{-x}\mu g/ml$ solvent actually means about $10^{-(x + 2)}\mu g$ of substance per glass rod tip. This 100-fold difference permits comparisons between concentrations on glass rods and filter paper.

The new stimulus–response function (Fig. 6) is more complex than expected. Only in the higher concentration range does the response rise with the logarithm of the stimulus concentration, while in the lower range this relationship is rarely found. The response to the isomers varies but

mostly stays behind Bombykol. The 10-*cis*-, 12-*trans* isomer usually is
second in order, followed by 10-*cis*, 12-*cis* and then 10-*trans*, 12-*trans*.
Relatively strong isomer-responses (see 10-*cis*, 12-*cis* in Fig. 6) appear fre-
quently at lower concentrations and cannot be adequately explained yet.

One remarkable feature of the Bombykol reaction curve is the enormous
concentration range covered by the antenna as a whole (Fig. 6). Equally
wide ranges of about 10^{12} are known only for the human eye and ear.
Even more striking is the Bombykol response curve. From threshold below
$10^{-10}\mu g$ to about $10^{-4}\mu g$, the reaction is practically constant. With
higher concentrations the intensity of the reaction first rises slowly and then
at a very fast rate.

From this response of the antenna to Bombykol, it may be deduced that
the animal is not able to distinguish between different molecular densities
with great sensitivity in the lower concentration range. But density dis-
crimination apparently becomes excellent in the higher range. This re-
lationship now appears to fit ideally to the requirements of a male moth in
nature. Perhaps a male, several hundred meters downwind from a female,
just " catches " with its olfactory receptors the minimum number of lure
molecules necessary to elicit excitation. This stimulus works as a signal to
induce or reinforce a positive anemotaxis (Schwinck, 1954) and thus
eventually leads the male to the neighbourhood of the female. During this
approach, the olfactory receptors of the male will constantly give the same
low level information to the brain, keeping him moving upwind. Odour
concentration differences due to lack of laminarity in the air stream will
rarely be large enough to change the information output of the olfactory
receptors. This could be very important, since the male might otherwise
deviate from the upwind direction in pursuit of every meaningless concen-
tration increase of lure odour. Finally, rather suddenly when the odour
concentration indicates the neighbourhood of the quietly waiting female,
an olfactory orientation primarily due to intensity discrimination may be
accomplished.

In support of this hypothesis is the following observation. Dissected
glands of females are electrophysiologically as effective as filter paper hold-
ing between $10^{-2}\mu g$ and $10\mu g$ of Bombykol. This concentration range is
exactly where the gradient of the curve (Fig. 6) is steepest and where odour
intensity discrimination is expected to be optimal. The stimulus–response
function of the antenna stimulated with Bombykol thus appears to ad-
mirably fulfil those requirements which may best serve the male in the
field.

Adaptation has not been extensively studied yet. However, it probably
is the main cause of the hysteresis curve obtained in an experiment where
the antenna was stimulated with ascending Bombykol concentrations first
and descending concentrations afterwards (Fig. 7). Reference stimuli of

low but effective concentration, used briefly after a strong stimulus, either failed completely or elicited weaker responses than before strong stimulus application. A reference stimulus is again as potent only after 5–10 min of recovery are allowed. During the application of highly concentrated stimuli, very often the EAG plateau is unsteady. This may also be partially the result of an interaction between degree of stimulation and rate of adaptation.
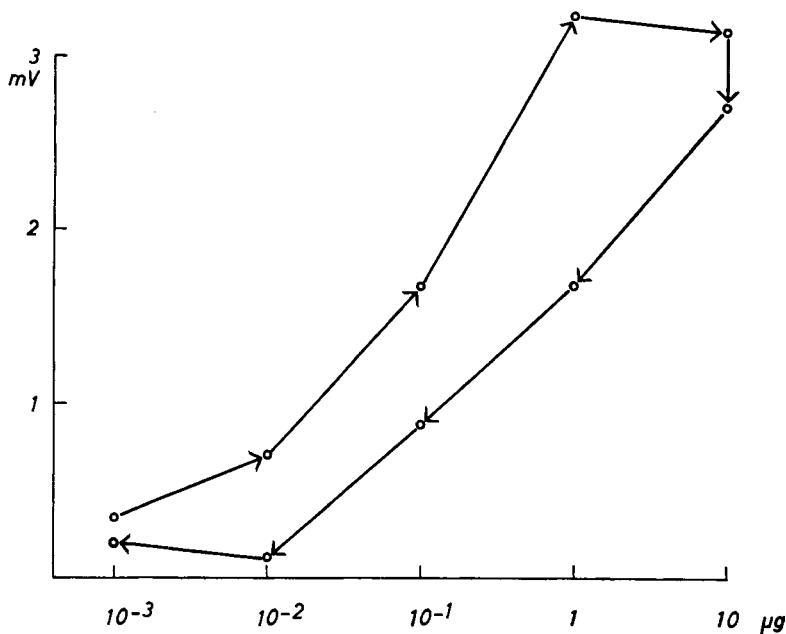
FIG. 7. The stimulus-response curve of *Bombyx* males to ascending and descending concentrations of hexadeca-10-*cis*, 12-*trans*-dien-ol-(1). Ordinate : EAG-Amplitude (from Block, Boeckh and Schneider, unpublished).

The threshold differences between Bombykol and its isomers are extremely interesting. The architecture of the molecule (Fig. 8) is obviously of decisive importance for olfactory stimulation. When we checked the isomers electrophysiologically, we usually found that the threshold difference between the " true " lure alcohol and the isomers was smaller than expected from the results of behaviour tests.

Except for a few cases where one of the isomers (mostly the 10-*cis*, 12-*cis*-substance) was apparently more potent than Bombykol (Fig. 6), the isomers stayed behind the Bombykol response. In some tests the curves of at least three of the four isomers of the hexadecadienol met in the higher concentration range.

All these variations are partially the results of too few data. Since the experiments are still in progress, more conclusive results are anticipated.

On the other hand, the variability may also derive from specific physio-
logical conditions due to the location of the recording electrode. We still
do not know whether only a rather small group of olfactory nerve cells near
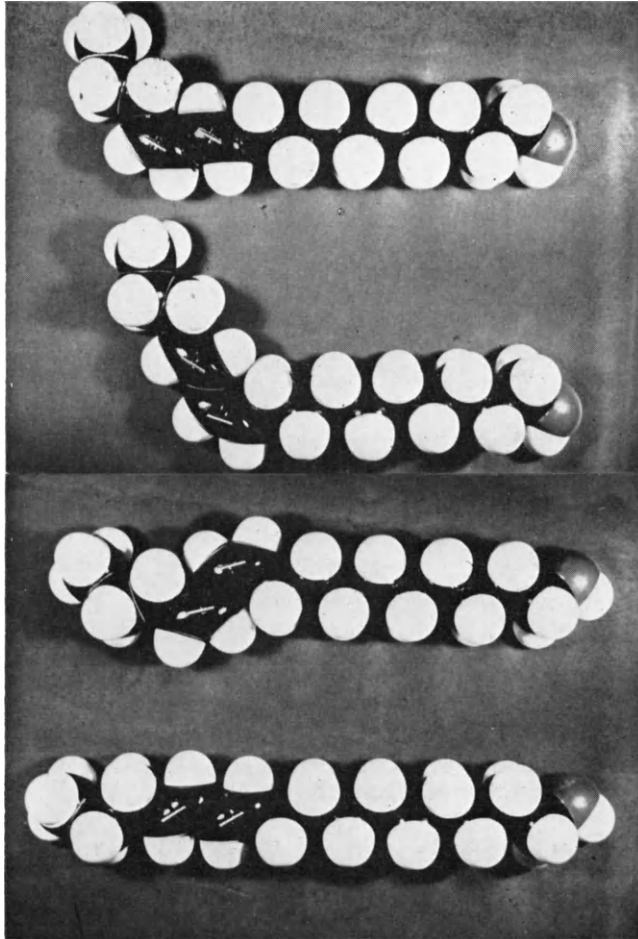
FIG. 8. Three-dimensional models of the four geometrical isomers of hexa-
decadienol. From above to below : 10-*trans*, 12-*cis* ; 10-*cis*, 12-*trans* ; 10-*cis*,
12-*cis* ; 12-*trans*, 12-*trans*. C-atoms are black ; H-atoms are white ; O-atoms
are grey (courtesy of Dr. E. Hecker).

the electrode tip is mainly responsible for the EAG. If this is the case,
response fluctuations, sensitivity shifts, and even the higher sensitivity to
an isomer could be explained by physiological differences of the receptor
cells from which the electrode is recording. The number of cells cannot
be too small, however, since we usually record with electrodes so wide that

impulses are rarely picked up. Recording from only a very few cells in the sensory epithelium (see Schneider, 1957) will bring nerve impulses of rather large size into the picture.

Similar electrophysiological experiments to the ones described with *Bombyx* have been done recently on a smaller scale with the gypsy moth,
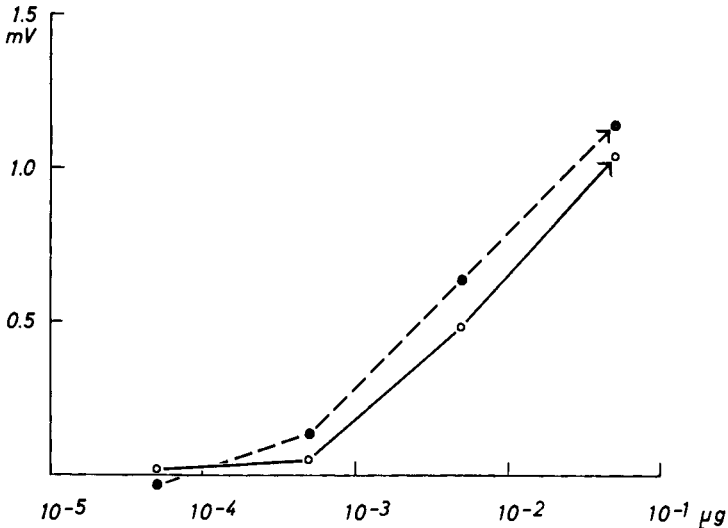
FIG. 9. The electrophysiological (EAG) stimulus-response curve of *Porthetria dispar* males to the optical isomers of acetoxyhexadecenol. The broken line represents the response curve to the *d*-isomer ; the solid line represents the response curve to the *l*-isomer. Illustrated is a typical experiment performed with one animal and one electrode position (from Boeckh and Schneider, unpublished).

Porthetria dispar (Block and Schneider, unpublished). As far as our information goes, the situation is much less complex than with *Bombyx*. The *Porthetria* male antenna studied with the EAG method shows the expected stimulus–response curve (Fig. 9). It rises with ascending concentrations first slowly and more sharply later. A saturation effect has not been observed, even with pure attractant in the highest concentration tested.

The gypsy moth sexual attracting substance is optically active and exists in a *d*- or dextrorotatory and an *l*- or levorotatory form. No activity differences between the two isomers could be found when checked with the EAG method. The same also seems to be true behaviourally since brief but informative tests showed little difference between the *d*- and the *l*-form. Also, we compared freshly dissected female glands of *Porthetria dispar* with known concentrations of lure substances on paper and found that the gland corresponds to about 0.05μg of attractant on paper.

Usually the sexual lure substances of moths are called species-specific.

That this is not necessarily so has already been demonstrated by behavioural tests (for literature see Schneider, 1962). Our information indicates that specificity may only reach to the family or sub-family level, as in the case of the Saturniids (Schneider, 1962). However, there is no EAG-response above control when the *Bombyx* male antenna is stimulated with the *Porthetria* lure gland or natural or synthetic substances and vice versa. While *Porthetria* males in behavioural tests are completely uneffected by virgin *Bombyx* females, the reverse is not true. The *Bombyx* males give a weak but definite response to the other species. Unfortunately, it was not yet possible to compare electrophysiologically the two well known *Porthetria* species, dispar and monacha. Behaviour tests by Schwinck (1955) demonstrated clearly that females of both species attract the males of the other.

While we did not expect a female moth to show any motor reaction when confronted with its own lure substance in high concentration, it was surprising to find that the *Bombyx* female antenna gave no EAG response to the gland or to synthesized Bombykol. Since such a female antenna was able to react to other odorous substances in the same way as the male antenna, it was concluded that the female moth does not have the specific receptor type for detecting its own perfume. Humans also cannot detect Bombykol, no matter how high the attractant concentration. The inability of the female moth to identify its own lure substance was also found in the Saturniids (Schneider, 1962) and more recently for the gypsy moth. The antennae of the female moths do not even have receptors to detect substances of the same " class " of attracting compounds. None of the female glands of *Bombyx* and of the Saturniids studied so far had an effect upon any of the antennae of the female donors. Recently we checked the antennae of female *Bombyx mori, Porthetria dispar* and *Antheraea pernyi* (Saturniidae) during stimulation with the female lure glands of all three females and could not record olfactory EAG's, although other scents affected the antennae (Block and Schneider, unpublished).

The threshold concentrations, given in absolute amounts of lure substance, make it possible to calculate the number of molecules expected on the glass rods or filter papers used as stimulus sources. At the lowest Bombykol threshold determined with the EAG method ($10^{-10}\mu$g on the 144 mm^2 piece of filter paper) there are only 10^5 molecules on the paper. The vapour pressure of Bombykol, although not yet determined, is probably not very high. Therefore, only a fraction of the available molecules actually leaves the filter paper during the stimulation time of 1–2 sec. These molecules are carried in a certain volume of air (3 l of air/min) which streams over the antenna. Without knowledge of the vapour pressure, it is impossible to make any precise calculations of the Bombykol-density at threshold, but it seems plausible to think of a rather small

number. This number must of course be great enough to ensure excitation in the receptor loci.

While a concentration of $10^{-10}\mu g$ on filter paper could possibly still elicit a response of the whole animal or its sense organs, concentration of less than $10^{-12}\mu g$ in positive behavioural tests (Butenandt *et al.*, 1959, 1960, 1961, and Fig. 5) appear to be " dangerously " low. To understand this problem it is necessary to recognize that the only way to prepare descending concentrations is to dilute in the usual manner. Thus, all the figures given in $\mu g/ml$ solvent or in μg on filter paper rely on the simple method of diluting stock solutions. To date we do not have a better way or, indeed, a method to check the actual amount of substance in the solvent. Hecker (unpublished) thought that an assembling of attractant molecules in the surface layer of the solvent may occur. If so, the amount of molecules attached to a glass rod would be higher than calculated. However, this explanation did not receive experimental support, because tests done with lure substance diluted in different solvents always gave the same very low threshold for Bombykol.

Finally, the potency of female glands was compared electrophysiologically with known amounts of lure substances. The normal glands of virgin *Bombyx* females are as effective as a piece of filter paper holding between $10^{-2}\mu g$ and $10\mu g$ of Bombykol. The amount of Bombykol that the chemists were able to isolate per female was about $10^{-2}\mu g$. The chemically determined amount of *d*-isomer per female gland for *Porthetria*, $5 \times 10^{-2}\mu g$, was also found to be in excellent agreement when checked experimentally.

While these results are indicative, it is necessary to point out that the paper piece or glass rod contaminated with the lure substance are only finite reservoirs, eventually depleted by evaporation or oxidation. The intact female gland, however, is a dynamic living system. It is a " factory ", producing the lure substance in certain cells and then allowing the odour to evaporate. Since the chemists reported that saponification of a certain fraction of the extracted material increased the activity, it is probable that part of the lure alcohol is stored in the gland in esterified form. However, this is really all we can say. For a number of reasons it is neither possible to estimate the number of molecules leaving the surface of the gland per unit time nor the amount of substance present in the gland at a certain moment. Consequently, the amount of lure substance a female produces over its life span of a few days remains unknown.

Biologically active substances transmitting information between individuals of the same species are called " *pheromones* " (Karlson and Lüscher, 1959 ; Karlson, 1960). Clearly the sexual attracting substances belong to this class. The pheromonereceptors may be compared analogously with a group of very intensively studied " chemoreceptors ", the synapses. Here for example a cholinergic pre-fibre (Fig. 10) produces

transmitter substance which, in the post-fibre, elicits membrane effects. In the synapse, the transmitter (e.g. acetylcholine) has to bridge over a synaptic cleft of 500Å at a maximum ; in *Bombyx* the " transmitter " (Bombykol) has to bridge over a very much larger distance. It is not clear how far reaching such a comparison is, because the properties of the

pre-fiber	Acetylcholine →	post-fiber
EMITTER	TRANSMITTER	RECEPTOR
Bombyx female lure gland cell	Bombykol →	Bombyx male olfactory receptor cell
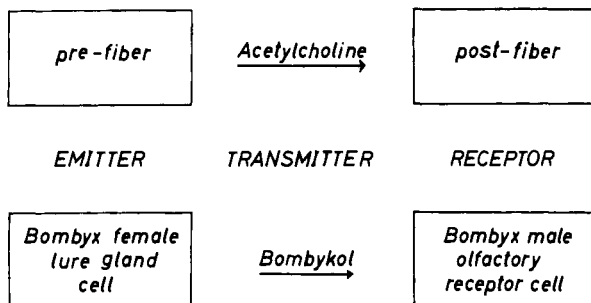
FIG. 10. Chemical information transfer in two highly specialized systems : (1) pre- and postsynaptic cholinergic nerve fibres ; (2) *Bombyx* female and male.

post-synaptic membrane and the olfactory receptor membrane have not been sufficiently worked out. Our knowledge of metabolic processes involved in the production and breakdown of synaptic transmitters is much more extensive than our knowledge of sexual attraction in *Bombyx* and *Porthetria*, where only the transmitter is known with certainty.

CONCLUSION

With electrophysiological methods it was possible to work out some details of olfactory receptor function in a few coleoptera and lepidoptera. Single unit olfactory receptors studied so far are of the sensillum basiconicum type. The first detectable electrical reaction of the receptor is a slow, graded receptor potential. Depolarization of the receptor membrane and nerve impulses are observed. In beetles at least, the receptor potential due to certain odours may be hyper-polarization which consequently blocks impulse generation.

Summated receptor potentials of a number of simultaneously active olfactory units are probably the main cause of a slow potential called the " electroantennogram ". Using this response as a test, the effects of sexual attractants on antennae of male *Porthetria dispar* and *Bombyx mori* have been checked over a wide concentration range. The response as the logarithm of the stimulating concentration rises over a certain range. In *Bombyx*, however, there is only a slight increase of response between 10^{-10} and $10^{-4}\mu$g of lure substance. In this range the animal may not be

able to make highly sensitive intensity discriminations.   But such dis-
criminations must be optional in the higher range, where response rises
steeply with concentration.   These higher concentrations are about equal
to the experimentally determined output of a female gland.   The *Bombyx*
sex attractant (Bombykol) is usually more effective than its geometrical
isomers.   The *d-* and *l-*form of the *Porthetria* sex attractant, as well as the
dl racemic mixture, are equally effective.

In no case studied so far did the attracting substances of any of the
female moths elicit a response in the antennae of any of the female of
these same species.

The lure substance of *Bombyx* and *Porthetria* did not elicit electrical
responses in cross-stimulation experiments.   However, *Bombyx* males
react with weak electrical responses to some glands of the Saturniids and
with weak behavioural responses to females of *Porthetria* and several
Saturniids.   In one of the Saturniid-subfamilies no species or genus
specificity of the attracting substances could be found.

Since the present state of our knowledge on many aspects of insect
olfaction is still very scant, it is perhaps worthwhile to define some areas
in which intensive investigation is necessary :

1. Determination of the identity and physiological range of the receptor
    cells responsible for the different chemical modalities as well as sub-
    sequent analysis of the fine structure and chemical composition of
    the receptor cell membrane.

2. Further elucidation of the sequence of processes taking place in the
    primary sensory cell under the influence of stimulating molecules.
    For the electro-physiologist this involves a detailed study of receptor
    membrane responses and generation of nerve impulses.   The trans-
    duction of " chemical energy " into graded potentials also has to be
    clarified.

3. Analysis of the structure and biochemistry of the lure gland.   This
    " emitting " system can be compared analogously to the presynaptic
    nerve fibre.   Bombykol is then the transmitter substance, eliciting
    electrogenesis in the olfactory receptor membrane (see Fig. 10).

4. Detailed study of the way in which an insect orients under the
    influence of olfactory stimuli.   Here information from the olfactory
    sense must be integrated with information from other sensory
    modalities.

supplied the *Porthetria*-attracting substances.   Dr. A. Ruperez (Madrid) generously provided the *Porthetria* pupae.

The author is indebted to Drs. B. C. Block (Williamsport) and J. Boeckh (Munich) for many constructive discussions during the preparation of the manuscript.

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THE FINE STRUCTURE OF THE OLFACTORY
RECEPTORS OF THE BLOWFLY*

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INTRODUCTION

The principal site of olfactory receptors in the blowfly *Phormia regina*, as in most insects, is the antennae. It has been demonstrated experimentally that the blowfly is able to perceive a variety of odors, including those of the normal aliphatic alcohols and aldehydes and a number of natural products, through the medium of antennal receptors (Dethier, Hackly, and Wagner-Jauregg, 1952 ; Dethier and Yost, 1952 ; Dethier, 1952, 1954, 1961). Furthermore, action potentials in response to stimulation by 0.01 M aqueous solutions of NH_4Cl and $NaCl$ have been recorded from these receptors (Wolbarsht and Dethier, 1958). Nevertheless, the identity of the olfactory receptors is in doubt and their exact structure unknown even though a number of histological studies of the dipterous antennae have been made (e.g. Liebermann, 1926).

Establishing the identity of the olfactory receptors is made difficult by the great numerical density of receptors, the variety of forms, and the minute size of all. By studying all types of antennal receptors with the electronmicroscope it has been possible to gain some idea as to the nature of the olfactory receptors because the basic features of all are strikingly similar.

MATERIAL AND METHODS

Antennae were removed from one-day-old flies in insect Ringers solution, placed in a chilled solution of 1 per cent s-collidine-buffered osmic acid to which had been added 0.045 g/ml of sucrose, and stored at 2°–4°C for 2–4 hr. Dehydration was carried out in graded alcohols. The final embedding mixture consisted of 90 parts butyl- and 10 parts methylmethacrylate. Two per cent Luperco CDB was employed to initiate polymerization. Polymerization was completed in gelatin capsules placed

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under ultraviolet light for 48 hr. Sections were cut with glass knives in a Porter-Blum and LKB ultramicrotome. Micrographs were taken with RCA-EMU-Z and Akashi TRS-50E electronmicroscopes at magnifications of 1000 to 16,000 times.

Receptors were also studied with a phase contrast microscope and after staining with methylene blue according to the method of Grabowski and Dethier (1954) and with Holmes' silver technique (Larsen, 1960).

ANTENNAL SENSILLA

The majority of sensory structures in insects (internal proprioceptors excepted) consist of a cuticular covering, which is a modified portion of the general body cuticula, one to approximately fifty bipolar neurons whose dendrites are associated with the cuticular covering and whose axons pass into the central nervous system, and three or more accessory epidermal cells which have no direct part in reception. A unit consisting of these components is termed a sensillum. The individual neuron is the receptor. A single sensillum may contain a number of receptors, each of which mediates a different modality (e.g. a labellar hair, which houses a water receptor, a sugar receptor, a salt receptor, and a mechanoreceptor).

The sensilla of the antenna, among which are numbered the olfactory receptors, are situated on the fleshy apical segment. Neither the basal segment nor the conspicuous plumose arista bear olfactory receptors. The sensilla are present on the entire surface of the segment and within large pits which are a conspicuous feature of the antenna.

Antennal pits are confined to the dorsal, ventral, and inner lateral distal half of the segment, this area being unpigmented. The remainder of the segment is fuscous. There is some variation in the number, size, and exact location of pits from one individual to the next, but in general the males possess from 9 to 11 and the females from 11 to 16. Some pits are simple, pocket-like cavities (Plate I, Fig. 5) ; others are multichambered (Plate I, Fig. 3). Their orifices are ringed with short, stout, spinous projections of the cuticle. Within the pits are found three types of sensilla : thin-walled pegs, thick-walled pegs, and coronal pegs, so called because of their crown-like appearance in transverse section (Plate I, Fig. 3). Only one type of sensillum occurs in a pit.

The surface of the antenna is thickly clothed with non-innervated spines with which the numerous sensilla are interspersed (Plate III, Fig. 3). The sensilla are of three general types : pointed, tapering, thick-walled pegs (Plate III, Fig. 1) ; short, rounded, thin-walled pegs (Plate III, Fig. 1) ; and minute stellate pegs (Plate III, Fig. 4). The thick-walled pegs, the most numerous type, are more or less evenly distributed over the entire surface of the segment, while the thin-walled pegs are absent or sparse on

the tip. The distribution of the third type is not known because it has been seen in only a few electronmicrographs.

One or more of these three types of sensillum is obviously olfactory. All possess many features in common. These are : a hollow cuticular peg all or part of whose wall is extremely thin and very possibly perforated ; a cuticular sheath or tube, the scolopoid sheath, extending from the basal region of the peg a variable distance down into the underlying tissue ; one to four deep-lying bipolar neurons whose dendrites pass into the scolopoid sheath thence into the lumen of the peg. Since there are many hundred-fold more cell bodies associated with the pegs than there are axons in the antennal nerve, and since synapses do not occur in the antenna, it follows that there is extensive fusion of axons. Axonal fusion is a common feature of the insect sensory system.

THIN-WALLED PEGS IN PITS (PLATES I AND II)

These pegs, characteristic of the more proximal antennal pits, are delicate rounded, structures averaging 13μ in length. In cross section they are circular except when cut slightly obliquely. The average diameter is 1.5-1.8μ. The cuticular wall at its thinnest point averages 0.08μ.

The number of neurons associated with each peg varies from one to two. Their cell bodies lie 15 or more microns below the base of the pegs. Their dendrites upon approaching the basal region of the peg enter the scolopoid sheath, which appears to fuse with the walls of the peg as it extends into it. Within the peg the dendrites completely fill the lumen.

A conspicuous feature of the cuticular walls of the peg is the indentations in its surface. Each peg possesses from 180 to 360, which constitute from 7–14 per cent of its surface. These are minute circular pits whose orifices are narrower (0.035μ) than the basal chamber (0.088μ) (Plate II, Fig. 1). It is difficult to ascertain whether or not these pits are indeed perforations, although this is the most likely interpretation. In any event, the electron-dense outer layer of the cuticle does not enter the openings. At the bottom of each opening is a material that is not part of the cuticle and seems to be continuous with the sheets and nets of tissue filling the lumen. At the base of the openings, however, and only there, the material occurs in the form of fine parallel strands. In cross sections these appear as round tubules.

THICK-WALLED PEGS IN PITS (PLATE I, FIG. 4)

These differ from the foregoing principally in possessing a thicker wall at the base and being more acutely pointed. Characteristically they are found in the more distal antennal pits. They also are innervated by one or two cells. When two neurons are present, the scolopoid sheath is compartmentalized to accommodate the two dendrites (Plate I, Fig. 4).

CORONAL PEGS IN PITS (PLATE I, FIGS. 1 AND 3)

These beautifully ornate pegs have not yet been identified with the light microscope, but in electronmicrographs appear in cross section as nearly perfect crowns with projections that usually number twelve. Longitudinal sections reveal that these projections are extensions of the peg wall which proceed outward at right angles to the long axis of the peg but subsequently bend down to hang parallel to the long axis. Accordingly, cross sections sometimes appear as smooth circles surrounded by a ring of dots. The pegs average 4μ in length and 0.5–0.8μ in basal diameter. They appear to be innervated by a single neuron, and no perforations have been discerned in their walls.

THIN-WALLED SURFACE PEGS (PLATE III)

These pegs average 25μ in length and are identical in nearly every respect with the thin-walled pegs found in pits.

THICK-WALLED SURFACE PEGS
(PLATE III, FIGS. 1 AND 3, PLATE IV)

These, the most common sensilla on the antennae, vary considerably in length. The average length is 83μ. The average basal diameter is 8μ. All of these taper more than the thin-walled sensilla. Some are straight and acutely pointed while others, usually the longer ones, are gracefully curved. The wall at the base may be from one-quarter to one-half as thick as the diameter of the lumen, but it becomes rapidly thinner as the peg tapers to its tip.

Each peg is innervated by one to four neurons whose cell bodies lie at a considerable distance beneath the surface. The scolopoid sheath is scalloped to accommodate the various number of dendrites (Plate IV, Figs. 1–4). As in the thin-walled pegs, the dendrites fill the lumen. The walls of the peg also contain the pits already described.

STELLATE SURFACE PEGS
(PLATE III, FIGS. 3–5, PLATE IV, FIG. 5)

The length of these small pegs is not known since they have been seen only in cross section. The basal diameter is about 1.2μ. The peg is characterized by much cuticular fluting and internal cuticular radii which suggest that the scolopoid sheath may extend some distance into the lumen. The structure is best understood by reference to the illustrations. Like the coronal pegs, these are few in number and are unlikely candidates for the function of olfaction.

DISCUSSION

The thick- and thin-walled sensilla on the surface and in the pits are

the most numerous sensilla on the antenna.   One or more of these types
has an olfactory function.   Since among them the fine structure differs in
minor details only, it is possible to derive from their description an idea
of the structure of the olfactory receptors.

The olfactory sensillum may be described as a hollow cuticular peg
innervated by one to four bipolar neurons.   The dendrites of the neurons
extend into a cuticular scolopoid sheath which is partially compartment-
alized to accommodate a particular number of dendrites.   The sheath
appears to fuse with the base of the peg and terminate there as a discrete
structure.   There is a continuous lumen from sheath to tip of peg.   In this
respect the relationship of the sheath to the peg differs from that described
by Slifer *et al.* (1957, 1959) for the olfactory sensilla of grasshoppers.   In
the long thick-walled sensillum of grasshoppers the sheath extends to the
tip of the peg which thus is left open ; in the short thin-walled sensillum it
opens at the base of the peg.   In both instances, as the insect molts and a
new peg and sheath are secreted, the old one is discarded through the
opening.   Since the fly does not molt, it is not surprising that the scolopoid
sheath fails to open to the outside.

There is some diversity in the appearance of the neural material filling
the lumina of the pegs, but thus far it has not been possible to relegate a
particular internal appearance to a particular type of peg.   The interiors of
the dendrites are markedly reticulate as the cross-sections in Plate IV
illustrate.   In longitudinal section (Plate III, Fig. 21) this reticulum is
more clearly seen and within it there appear to be contorted tubules seen
here both in cross and longitudinal aspect.   The empty space between this
material and the wall of the peg probably results from shrinkage occuring
during fixation.   Higher magnifications (Plate II) emphasize the irregularity
of the reticulum and the marked tubular appearance (Figs. 4 and 6).   In
some sections the tubular aspect is absent (Plate II, Fig. 5) and the ground
material is more finely uniform.   In other sections (Plate I, Fig. 2, Plate III,
Fig. 5) a tubular construction is pronounced.

A marked tubular appearance suggests that the dendrite has subdivided
into many parallel branches.   Where evidence of tubules is absent the
appearance suggests that a single expanded dendrite fills the lumen.
Further work is required to clarify this point.   In the thick-walled sensillum
of the grasshopper the four or five neurons send their dendrites unbranched
to the top of the peg.   In the thin-walled sensillum of the grasshopper the
forty (average number) neurons possess dendrites which become multiple
branches within the lumen of the peg.   In the fly the dendrites, whether
branched or unbranched, occupy nearly all the space in the peg and are
closely appressed to its wall throughout.

The walls have a peculiar structure.   They are uniformly pitted or per-
forated with 180 to 350 openings.   These may actually be holes in the

cuticle such as occur in the grasshopper (Slifer *et al.*, 1959). As in the grasshopper, the epicuticle does not extend beyond the opening. At the base of the pit in flies, there is a tissue which appears as fine filaments aligned perpendicular to the surface. Similarly in the grasshopper there are about 24 filaments to an opening, and they extend well up into the opening. According to Slifer *et al.* (1959) these are actually terminations of the dendrites. It has not been possible to ascertain whether or not this is the case in the sensilla of flies, but because of the similarity in other respects it hardly seems likely that the fly and the grasshopper sensilla differ in so fundamental an aspect. In any case, whether these are parts of the dendrites or extra-dendritic material, the dendrites, either by branching or by expanding to fill the lumen present a rather large part of their surface to the outside.

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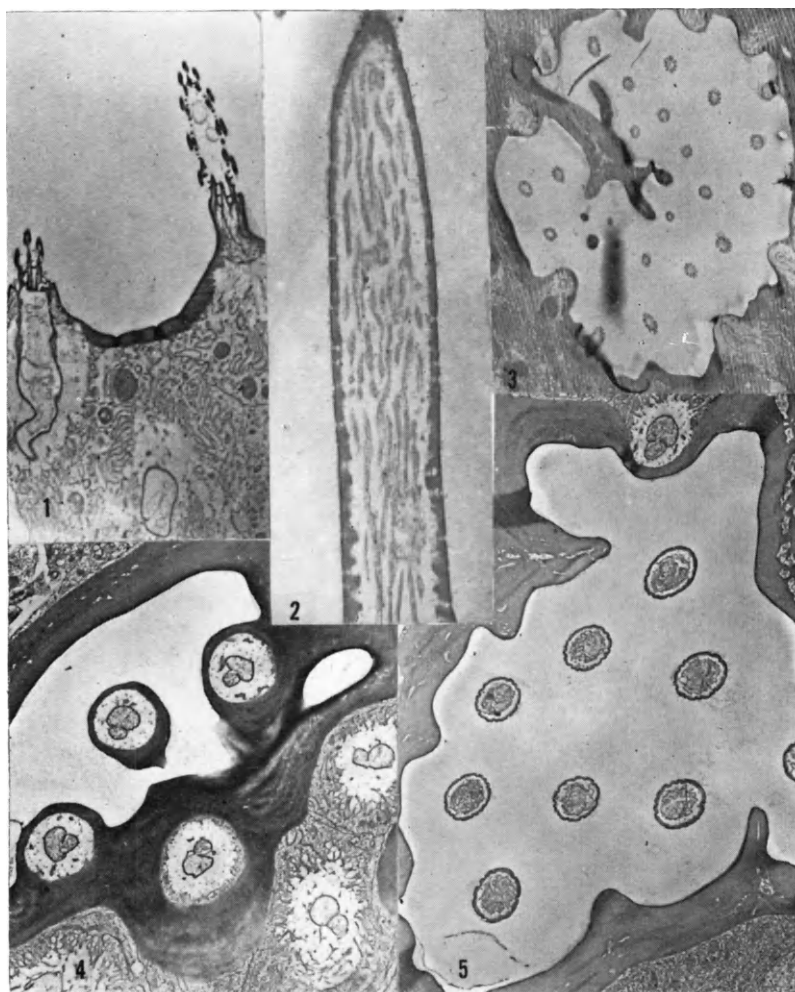
PLATE I. Pit sensilla on the antennae of the blowfly.

Fig. 1. Longitudinal section through two coronal sensilla. Note the large scolopoid sheath at the base of the left-hand sensillum ($\times$ 12,300).

Fig. 2. Longitudinal section through a thin-walled sensillum ($\times$ 14,000).

Fig. 3. Transverse section through a pit containing coronal sensilla ($\times$ 6000).

Fig. 4. Transverse section through thick-walled sensilla. Note the bipartite scolopoid sheath ($\times$ 49,000).

Fig. 5. Transverse section through a pit containing nine thin-walled sensilla ($\times$ 4900).
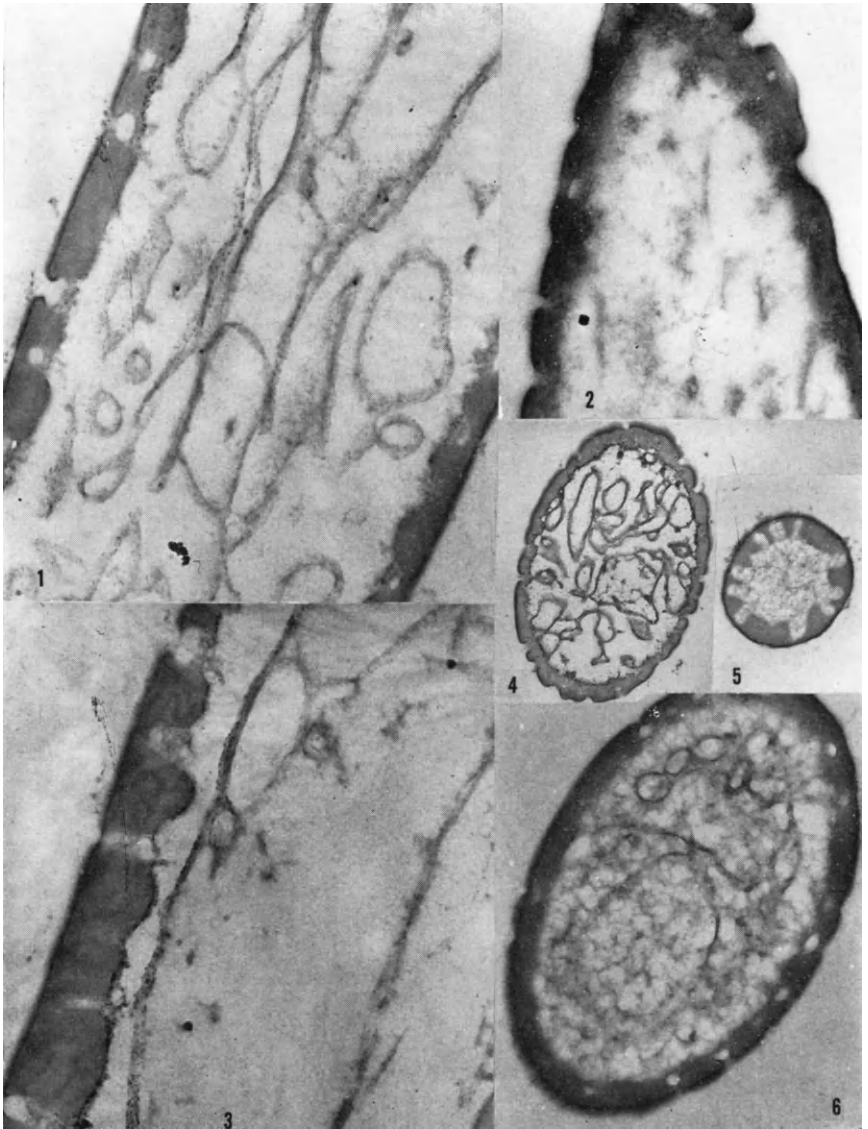
PLATE II. Pit sensilla on the antennae of the blowfly.

FIG. 1. Longitudinal section through a thin-walled sensillum. Note pits in cuticle ($\times$ 16,000).

FIG. 2. Longitudinal section through the tip of a thick-walled sensillum. Note even reticular appearance of material within pits which are seen in transverse section ($\times$ 16,000).

FIG. 3. Longitudinal section through a thin-walled sensillum ($\times$ 56,000).

FIG. 4. Transverse section through a thin-walled sensillum. Note pits on left where material within is seen in longitudinal section as parallel fibers or tubules and pits at top where the same material is seen in transverse section ($\times$ 28,000).

FIG. 5. Transverse section through the apical region of a thick-walled sensillum ($\times$ 14,000).

FIG. 6. Transverse section through a thin-walled sensillum ($\times$ 56,000).
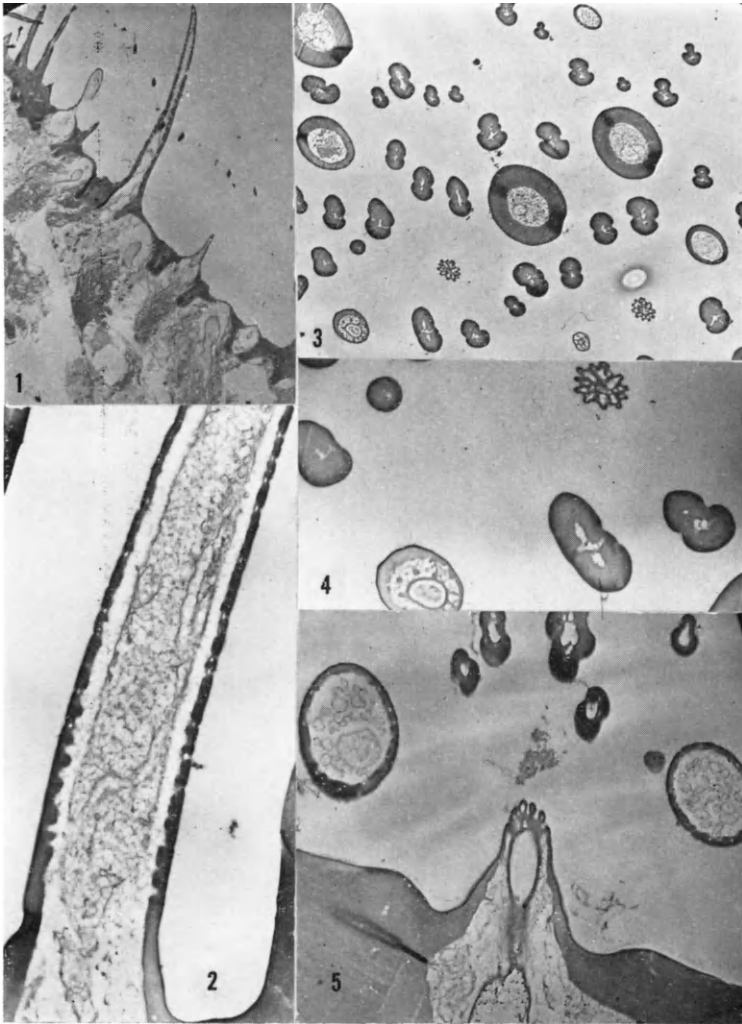
PLATE III. Surface sensilla on the antennae of the blowfly.

FIG. 1. Longitudinal section through a long thick-walled sensillum and a short, blunt thin-walled sensillum ($\times$ 6000).

FIG. 2. Longitudinal section through a thin-walled sensillum ($\times$ 14,000).

FIG. 3. Transverse section of five large thick-walled sensilla, one thin-walled sensillum (extreme top right), two stellate sensilla, and numerous non-innervated spines ($\times$ 4900).

FIG. 4. Same section as preceding enlarged to show some details of a stellate sensillum.

FIG. 5. Transverse section of two thin-walled sensilla and an oblique section of a stellate sensillum. Note the large scolopoid sheath at the base of the latter ($\times$ 9800).
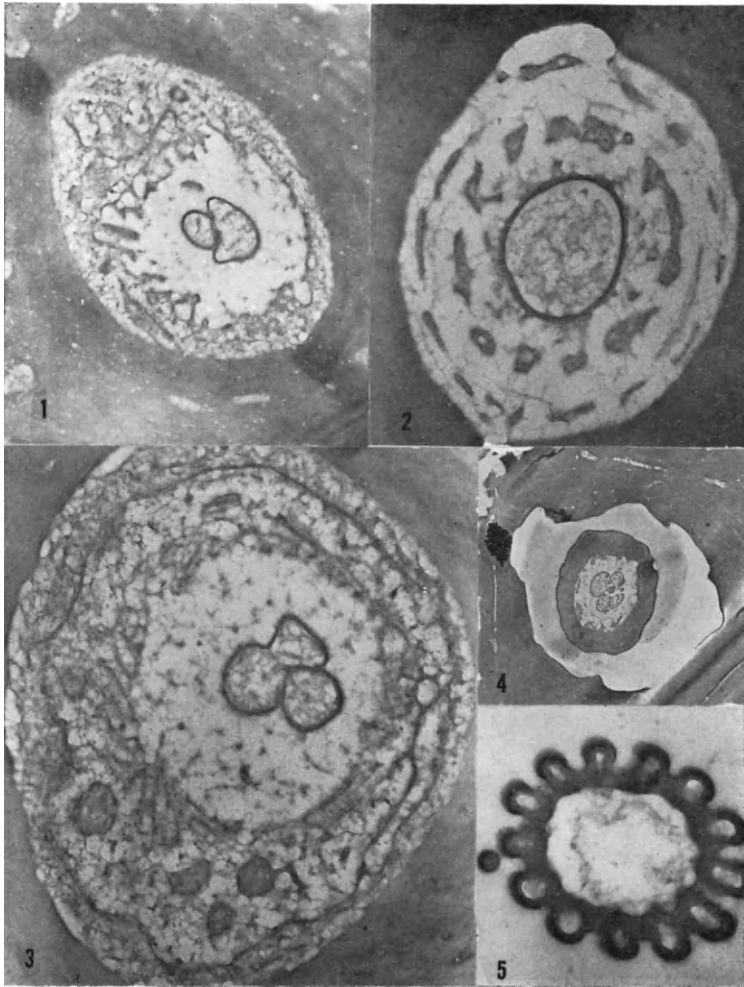
PLATE IV. Surface sensilla on the antennae of the blowfly.

FIG. 1. Transverse section through the base of a thick-walled sensillum showing a bipartite scolopoid sheath ($\times$ 28,000).

FIG. 2. Transverse section through the base of a thick-walled sensillum showing a tripartite scolopoid sheath ($\times$ 56,000).

FIG. 3. Transverse section through the base of a thick-walled sensillum showing a unitary scolopoid sheath ($\times$ 39,200).

FIG. 4. Transverse section through the base of a thick-walled sensillum showing a quadripartite scolopoid sheath ($\times$ 49,000).

FIG. 5. Transverse section through a stellate sensillum ($\times$ 56,000).

ON THE OLFACTORY SENSE OF BIRDS

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INTRODUCTION

" RERUM naturae scriptores non parum inter se dissentiunt circa avium olfactum. Sunt enim qui sensum hunc segnem admodum et obtusum in alotibus esse doceant, dum alii contra acri et exquisito Volucres olfacto praeditas esse contendunt. . . . " In spite of numerous investigations the inconsistencies in our knowledge on the olfactory sense of birds has not changed since Scarpa in 1789 so accurately described the knowledge of his time on this subject. I hope I will be able to show that these inconsistencies are due to peculiarities in the olfactory complexes in the birds. Mr. E. Fink has performed the first experiment under my guidance, and has assisted me with the others.

THE MORPHOLOGY OF THE OLFACTORY ORGAN OF THE BIRD

The nasal cavity of the bird is surrounded by the premaxillary, maxillary, nasal and palatine bones, and is also divided into two parts by the septum. The air passes through the outer openings and the primary and secondary Choana. The nostrils are small or even closed in the Steganopodes and Sphenisci. Each half of the nose is divided by three muscles : the lower (Praeconcha), the middle (Concha), and the upper (Postconcha) (Fig. 1). In many birds the last one is only a small bulge, or is entirely missing. There are olfactory sense cells in most birds, but only in the region of the Postconcha (Fig. 2). Depending upon the size of this region the number of olfactory cells varies very much indeed. The exact numbers are not known, in contrast to the mammals, and especially the dog. The structure of the avian olfactory region as basically similar to the mammalian one, with its olfactory cells, supporting cells, basal cells and the glands of Bowman.

The olfactory cell nerve fibres ascend to the Pars bulbaris in the Lobus olfactorius, where they end. The secondary olfactory pathways are less well developed in the birds than in the mammals (Fig. 3). A pathway to

FIG. 1. Cross section through the olfactory cavity of a one-year-old pigeon, haematoxylin Heid., 10 : 1.
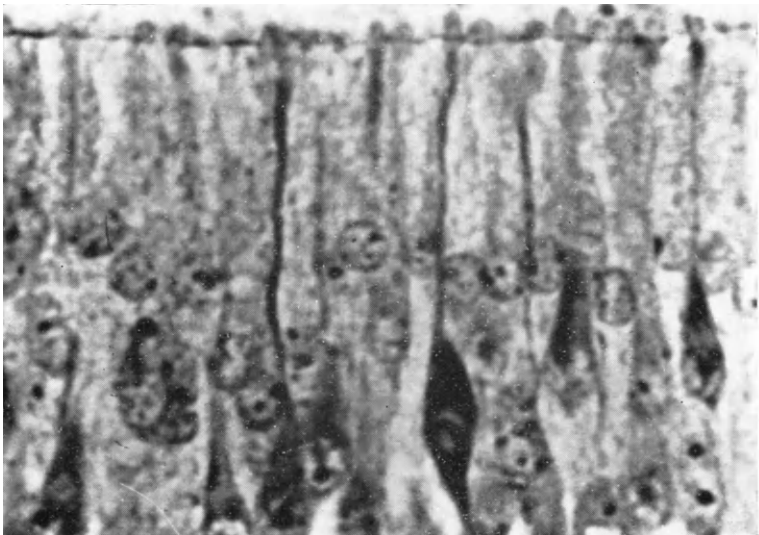
FIG. 2. Olfactory cells in regio olfactoria of a one-year-old pigeon. 6μ, iron haematoxylin, 900 : 1.
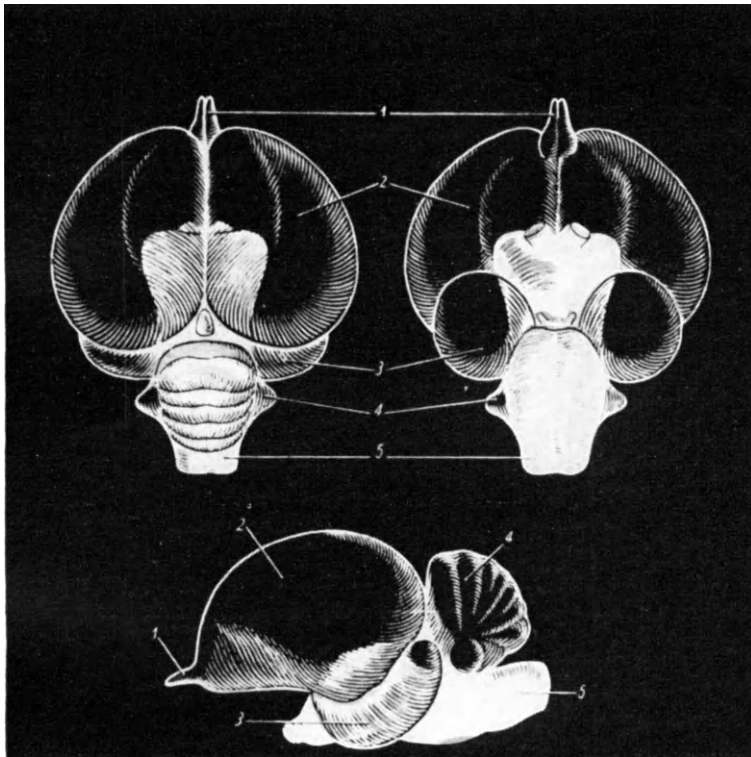
FIG. 3. Schematic picture of the avian brain, after Portman, 1950.
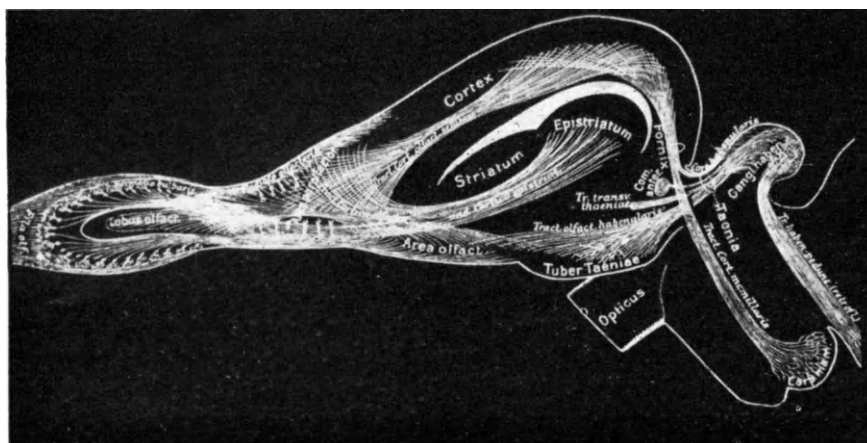
FIG. 4. Schematic picture of the saurian brain with olfactory pathways drawn in (after Edinger, 1900).

the diencephalon is the only well-developed one which is a circumstance of great importance for the interpretation of our experiments (Fig. 4). Judging from morphology and histology only, birds must have the sense of smell. As has already been stated, the experimental evidence on this topic is contradictory. Without attempting any comprehensive review, the following will show that even in recent years conflicting evidence has been published. Positive response to olfactory conditioning in robin redbreast (*Erithacus rubecula* L.), garden warbler (*Sylvia* spec.), thrush (*Turdus merula* L.), greenfinch (*Chloris chloris* L.), blue titmouse (*Parus caeruleus* L.) and Mallard (*Anas platyrryncha* L.) was obtained by Zahn (1935) and in robin redbreast by Wagner (1939). Contrarily, using the method of Bandurow and Laurin (1935), Walter (1943) could not evoke any olfactory conditioned reflexes in pigeon (*Columba livia* L.), while the Russian authors obtained positive results. Also Calvin (1957) using a similar method could not obtain positive results in pigeons. Michelsen (1959), however, has reported that two pigeons guided by their sense of smell only, were able to learn how to find food concealed in a complicated choice situation.

OLFACTORY LEARNING EXPERIMENTS WITH BIRDS

Our first experiments were also performed using pigeons in a three-choice apparatus. The animals could fly freely from the aviary to a box, from which there was one gate allowing them to get into a compartment containing food, while the other two gates were locked. This, however, the animals could not detect until they tried to pass through the gate (Fig. 5). The gates were closed with grids of a type used by carrier pigeon breeders. A pigeon having made the right choice was rewarded with food and then brought back to the aviary. The gate leading to the reward was marked by the smell of butyric acid on a filter paper in a test tube. As the three gates were enclosed and separated by Plexiglass plates, and a weak, continuous stream of air was caused to flow longitudinally through the apparatus, no smell could be detected, at least by a human nose, in the two locked gates, but only in the open one.

These experiments (Table 1), which include in all 1242 trials with seven male pigeons, do not prove the presence of an olfactory sense in pigeons, however. Even if in five pigeons the number of correct first choices is 33 per cent larger than for a random choice, this is not statistically significant. In three animals, however, there seems to be a tendency towards an increasing learning, which is shown by the higher percentage of correct choices in the last 30 trials. The experiment was discontinued, because no sure progress seemed to be possible.

The next experiment with three pigeons combined punishment in the form of NaCl polluted water with the odour of eugenol, against pure water

without odour.   The NaCl pollution was very effective in contrast to pollution with quinine sulfate as used by Zahn, which in a concentrated solution is not discriminated against by pigeons.   The water was offered to the pigeons in flat glass bowls, which were set in somewhat larger bowls, one of which contained the odorous substance.   Already in the first tests the pigeons observed even the minutest differences in the bowls such as
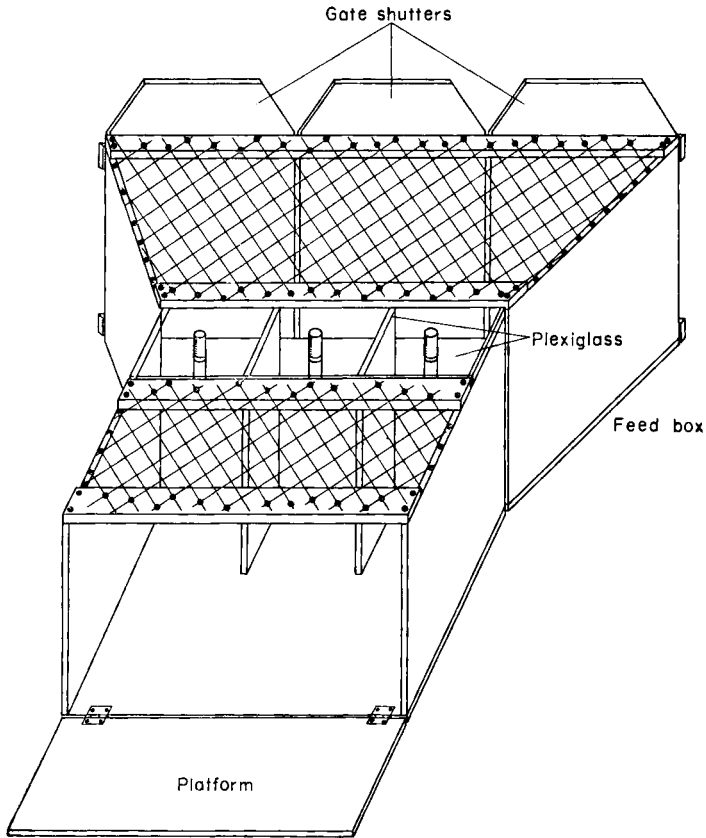
FIG. 5.   Three-choice apparatus for olfactory experiments on pigeons.

scratches or small air bubbles in the glass.   Thus their score was near 100 per cent correct choices necessitating a further series of 1024 trials using as similar bowls as possible, and switching them around irregularly, to avoid the possibility of optical orientation.   In this new series the score fell to 50 per cent, corresponding to random choices.   Though no statistically significant learning scores were obtained, still this series and later ones were important for the evaluation of earlier experiments, because it showed how easily the experimenter can be led astray by the very precise optical alertness of the birds.

In another series of 222 trials with a tame robin redbreast (*Erithacus rubecula* L.) using amyl acetate as a warning against NaCl polluted food (meal worm or commercial bird food) no statistically significant scores were obtained when all possibilities of optical orientation were avoided. In the first 20 trials a score of 90 per cent was obtained, due to insufficient avoidance of the possibility of optical discrimination.

TABLE 1. EXPERIMENT ON PIGEONS IN A THREE-CHOICE APPARATUS ; CONDITIONING WITH BUTYRIC ACID.

I. Pigeon no.	81	93	97	102	109	110	393
II. Total no. of trials	219	206	193	203	126	112	183
III. Correct choices	93	77	74	58	32	44	85
IV. Correct choices % Error	42.5 ±11	37.5 ±11	38.3 ±11	28.6 ±11	25.4 ±14	30.2 ±14	46.4 ±12
V. Correct choices in the last 30 trials % Error + 29, − 26%	62.8	33.3	46.7	23.3	33.3	43.3	50.0

In 1935 Zahn described an experiment in which a blue titmouse (*Parus caeruleus* L.) after only 10 trials was able to discriminate against contaminated food scented with Skatole. In a series of 334 trials with the same animal and the same odorous substance, we obtained a non-significant score (54 ± 9 per cent), using the same experimental set up as with the pigeons. Zahn reported that a thrush (*Turdus merula* L.) could be taught to distinguish between clove oil in polluted food and rose oil scented food. It was not possible for us to find an effective pollutive substance. Meal worms which have been kept in concentrated sodium chloride solution, tartaric acid or methanol were eaten without any hesitation. Instead, we used a punishment in the form of an electric shock at the bowl of rose oil while the bird obtained a reward in the form of a meal worm at the bowl with clove oil. In 285 trials the score was in the random range. Using optical marking instead of olfactory, 40 trials were enough to reach a high score. In the last 20 trials a score of 95 per cent was obtained.

Finally a series of experiments with geese in an apparatus similar to the one Michelsen (1959) used in his experiments with pigeons will be described. It was designed to teach the pigeons to correlate smell or lack of smell with colour signs. After a correct choice of a colour associated with the presence or absence of the odour of butyric acid, they were rewarded with food. The scores obtained in 691 trials with a male and 729 with a female goose were in the random range, while Michelsen's pigeons were able to correlate smell and colour correctly.

All those negative experiments of ours show that it is very difficult indeed

to teach birds to answer to an olfactory stimulus. A comparison of our results with those of Zahn shows that it is very easy to be deceived by the extraordinary power of optical discrimination of the birds. Our results are in accordance with the recent findings of most other authors.

The different species which we have tested all have an olfactory epithelium, which, at least in the pigeon, is not degenerate, as our own histological investigation has shown. Thus, there remains the existence of an olfactory organ without any demonstrable function. It was then thought that it might prove a fruitful line of research to look for spontaneous olfactory responses without any attempt at training. Because of the secondary olfactory pathways to the diencephalon, such spontaneous responses might be expected to involve vegetative functions.

SPONTANEOUS OLFACTORY RESPIRATION REACTIONS IN GEESE

The experimental animal used for further studies was the greylag goose (*Anser anser* L.). Seed-eaters do not have much use for an olfactory sense in their search for and choice of food. In contrast to this, geese mostly live on green plants, the safe choice of which surely is facilitated by a good sense of smell. Also the olfactory epithelium is well developed, and, because of the size of the animals, it has a large area, which is of some significance for the sense of smell (Neuhaus, 1957).
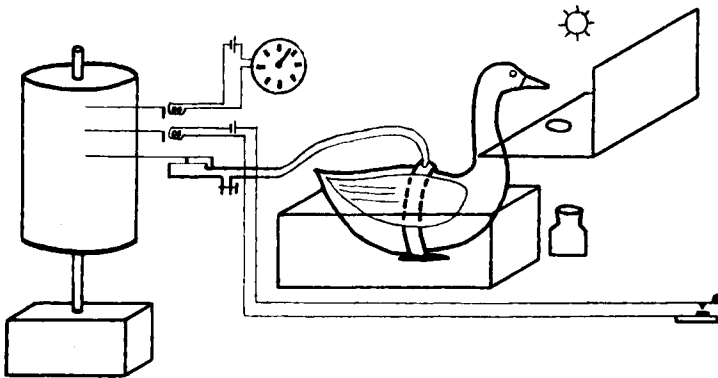
FIG. 6. Schematic picture of apparatus for measuring respiratory reactions to olfactory stimuli.

The vegetative reaction studied was respiration, which is easily influenced by all sorts of factors.

After the animals had got used to the experimenter they were made familiar with the apparatus, which permitted recording of the respiration without disturbing the animals (Fig. 6). The animal was seated comfortably in a roomy nest. With a belt behind the shoulder joint a flat thin-

walled 40 mm diameter rubber tube was held quite loosely around the animal. This respiration belt was connected to a Marey's capsule via a narrow bore rubber tube. The pen of the capsule recorded the pressure changes on a Kymograph. In order to avoid all nonrelevant stimuli, the experiments were performed in a darkened silent room. The little light needed for manipulation (a low-voltage lamp) was so situated that the animal could not perceive anything of the handling of the apparatus. The animal was in direct light, while all the manipulation took place in dim light screened from the animal.
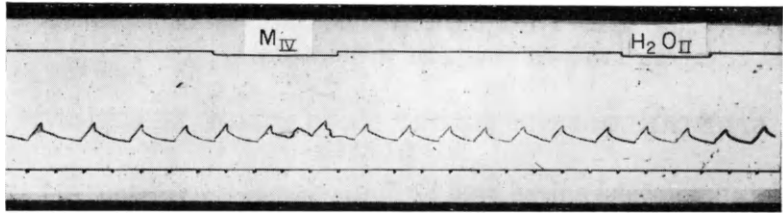
FIG. 7. Record of the respiration of goose No. 1. Ethyl mercaptan and water. (Time 5 sec).
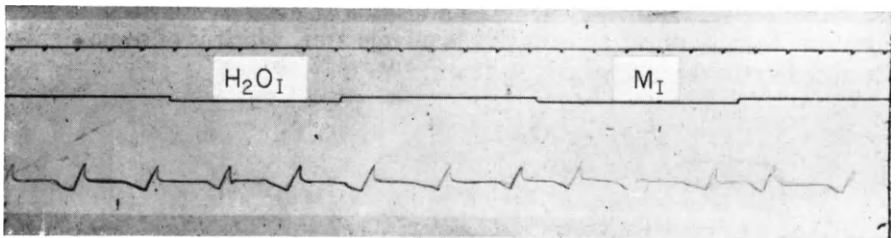
FIG. 8. Record of the respiration of goose No. 1. Water and ethyl mercaptan.

The recording system was filled with air and a period of calm, regular respiration was waited for. Then a vessel containing a concentrated odorous substance was quietly opened under the beak of the bird, and held there 20 to 40 sec. The animal could not see the manipulation or the vessel. As controls water vessels were introduced before, after, and between the odour-containing vessels. After each olfactory test the room was ventilated for 10 min to abolish any traces of the odorous substances. Figures 7–16 shows some recordings from two ganders and one goose. The regular respiration, i.e. a fairly fast inspiration, followed by an at first fast and later slow expiration—is usually changed by the introduction of the olfactory stimulus to a more irregular pattern. Frequently, the first inspiration shortened after the introduction of the stimulus and the second lengthened, or the respiratory pattern changed to a series of short puffs. The controls with water showed no such changes. The first pictures show

H_2O_I M_I

Head tilted
Swallowed
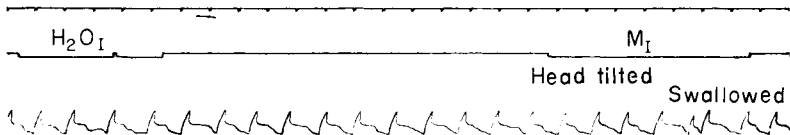
FIG. 9. Record of the respiration of goose No. 2. Ethyl mercaptan and water.

H_2O_I M
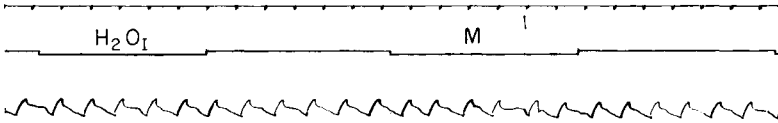
FIG. 10. Record of the respiration of goose No. 2. Water and ethyl mercaptan.

S_{IV}
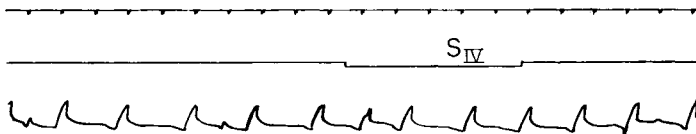
FIG. 11. Record of the respiration of goose No. 2. Skatole.

S_{IV}
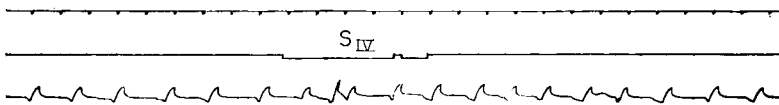
FIG. 12. Record of the respiration of goose No. 2. Skatole.

S^*
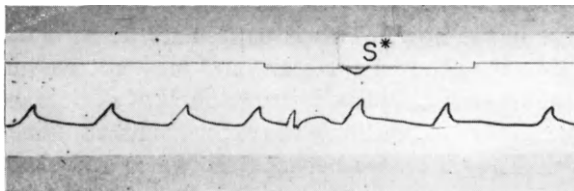
FIG. 13. Record of the respiration of goose No. 3. Skatole.

experiments with ethyl mercaptan, the others with skatole. Formaldehyde and a mixture of many aromatic substances were also tested. All substances gave positive responses.

All the results were not as obvious as those shown in the figures. Sometimes there were spontaneous variations in the respiration without any known causes, which could appear when a water control was tested. Sometimes it was impossible to avoid extraneous disturbances. Finally some of the responses were not quite clear. Thus the results had to be classified in three groups: (1) positive reaction to smell or to water, (2) absence of reaction, (3) doubtful.
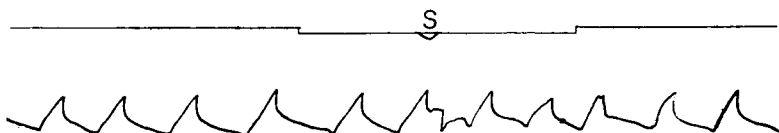
FIG. 14. Record of the respiration of goose No. 3. Skatole.
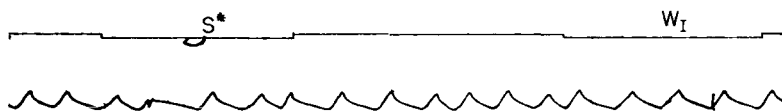
FIG. 15. Record of the respiration of goose No. 3. Skatole and water.
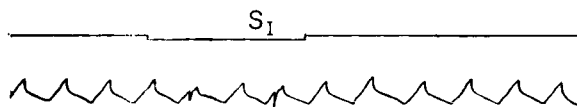
FIG. 16. Record of the respiration of goose No. 3. Skatole.

Under careful consideration of all critical points of view, Table 2 was set up. Skatole is the only odorous substance which has a " pure odour " in the sense of v. Skramlik (1924), while formaldehyde, ethyl mercaptan and also the aromatic mixture are " common odours ", which stimulate not only the olfactory receptors but also the free nerve endings of the trigeminal nerve in the nose. This is particularly true for formaldehyde. This classification is proposed for man, and may not be valid for birds. Thus it was thought that only the " pure odour " of skatole should be used for further discussion. Accordingly the experiments contained in Table 3 were performed. The difference between the water and skatole figures is large, but may be not immediately convincing. The X^2 test was used to investigate the probability for the frequencies in the three classes for water

TABLE 2. SPONTANEOUS REACTIONS TO OLFACTORY STIMULI OF GEESE

Animal	Test substance	Number of tests	Reaction		
			Positive	Negative	Doubtful
Gander 1	Ethyl mercaptan	11	7	3	1
	Formaldehyde	4	4	0	0
	Aromatic mixture	4	1	3	0
	Water	8	0	8	0
Goose	Ethyl mercaptan	27	16	6	5
	Skatole	26	9	10	7
	Water	95	26	57	12
Gander 2	Ethyl mercaptan	5	4	0	1
	Skatole	15	14	0	1
	Water	42	15	19	8

TABLE 3. SPONTANEOUS REACTIONS OF TWO GEESE TO SKATOLE AND WATER

Test substance	Number of tests	Reaction		
		Positive	Negative	Doubtful
Skatole	41	23	10	7
Water	145	41	84	20

Probability for a random result $p = 0.0007$.

and for formaldehyde to be samples from the same population. According to the formula

$$\chi^2 = \frac{1}{n \cdot n'} \sum \frac{(z_i \, n' - z_i' \, n)^2}{z_i + z_i'}$$

a value for χ^2 of 27 was obtained, corresponding to a probability of $p = 0.0007$. Thus it is proved that geese behave differently when exposed to skatole as compared to water. This can only mean that the geese have shown an olfactory response to skatole.

DISCUSSION OF THE RESULTS

The last experiment seems to contradict the first mentioned experiments. An explanation can be found in the central olfactory pathways. The secondary and tertiary olfactory fibre bundles of the birds are, in contrast to those in the mammals, little developed, particularly those ending in the

forebrain, for example in the *Epistriatum*. The pathways to the brain stem are more highly developed, that is the tractus olfacto-habenularis, ascending to the diencephalon. As the olfactory pathways to the forebrain are better developed in reptiles and particularly in mammals (Fig. 4) it is probable that they have reduced in birds, while the connections to the diencephalon have not. In the higher vertebrates the power of association is mainly restricted to the forebrain. Optical and acoustical pathways and nuclei are also well developed in birds, in accordance with their good learning power in these respects. As the histological structures necessary for olfactory learning are poorly developed in the bird, it seems probable that learning experiments based on olfaction would be difficult to perform successfully. Even in man, with his relatively poor sense of smell, the power of olfactory learning is probably smaller than in the dog with its much more sensitive olfaction. Because of the well-developed connections to the brain stem, on the other hand, spontaneous, particularly vegetative reactions of the bird seem quite possible.

These ideas are quite in accordance with our experimental results. It was not possible for us to teach birds of different species to respond to olfactory stimuli, which is in agreement with many other authors. On the other hand, our three geese spontaneously responded to strong olfactory stimuli with a changed respiratory rhythm. It is quite possible that there also occur other, less easily measured, vegetative reactions. These effects are surely a matter of nonconditioned reflexes, which are not, however, of the preciseness of the patellar reflex, because of the many synapses in the reflex chain. Presumably they have a biological purpose, which, however, is not clearly known at present. All in all it can be stated that in birds olfactory reactions are possible, corresponding to the available substrate of nerves and receptors, but that contrarily to most mammals olfactory responses of birds are inferior to visually, auditorily or mechanically conditioned responses.

<div align="center">SUMMARY</div>

With methods, partly intentionally in conformity with other authors, it was not possible to obtain any positive olfactory learning in pigeon, robin redbreast, blue titmouse, thrush, and goose.

Sometimes seemingly positive results were obtained, which always, however, could be attributed to optical perception. This result implies that perhaps the earlier authors have not sufficiently obviated the possibility of optical orientation. In spite of this a perception and elaboration is shown by the changed respiratory rhythm in response to an olfactory stimulus.

The difference in the results, on one hand the impossibility of obtaining olfactory learning response, while there are, on the other hand, the

spontaneous respiratory reactions, is explained by the fact that only those olfactory central pathways are well developed that end in the diencephalon. Thus olfactory association, which is necessary for successful learning, can take place only with difficulty.

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THE FUNDAMENTAL SUBSTRATES OF TASTE

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I

THE concept of a *substrate* in the domain of sensory physiology should include all those elements necessary for the perception of one single fundamental sensation, thus consisting of the receptor at one end and the perceiver at the other connected by a chain of neurones. Since there are four principal qualities of taste, it may be inferred that there are four gustatory substrates, each represented by a distinct group of uniformly working taste buds. It is well known that the four substrates have different sensitivities to gustatory substances. Most sensitive is the substrate of bitter, 10^{15} molecules of strychnine on the tongue being enough to evoke a definite sensation of bitter, while about 10^{20} molecules of sugar are needed to evoke a sweet sensation. Measured this way, the bitter substrate is one hundred thousand times more sensitive than the sweet one.

In this connection, the question has been raised whether there exists any correlation between the physiological sensitivity of a substrate and its pathological susceptibility to injury, because it is known that the bitter substrate which is the most sensitive, is also very susceptible to damage by ionizing radiation, while the other three less sensitive substrates are by far less susceptible to such damage. The pathological sensitivity of the four substrates may also be tested by their susceptibility to anaesthetics. Since only occasional observations have been made in this regard my collaborators and I have decided to perform a systematic investigation which will be described in this paper.

The proposed problem could be elucidated in a quantitative way by measuring the thresholds of the four gustatory substrates, before, and at regular intervals after the application of anaesthetic solutions to the tongue. The ratio of threshold after to threshold before anaesthesia constitutes a numerical measure of the effect of the anaesthetic. A high value indicates a strong anaesthesia, while a value less than one implies that the anaesthetic has caused hypersensitivity.

II

As gustatory test substances were used quinine hydrochloride, sodium

chloride, tartaric acid and cane sugar. The anaesthetics are listed below according to their chemical structure :

Anaesthesin, Orthoform, Cycloform, Anaestheform, Subcutin, Novocain, Tutocain, Larocain, Pantocain, Stovain, Alypin, 1-Cocain, Psicain *old*, Psicain *new* and Tropacocain in 0.30 M solutions, Eucain β in 0.176 M solution and Holocain, Diocain, Acoin, Percain and Eucupin were used in 0.03 M solutions. The latter substances had too low a solubility to permit 0.30 M solutions to be made. Anaesthesin, Orthoform and Cyclo-form had to be dissolved in 40–50 per cent alcohol.

The gustatory surface in the mouth was brushed with the anaesthetic solution for 3 min, in the following order : first the tip of the tongue, then the sides, the middle, the bottom and finally the soft palate.

The thresholds were measured before and 1, 5, 10 and 30 min after the application of the anaesthetic. These measurements were always made in the same order : Sodium chloride, tartaric acid, and cane sugar, leaving quinine to the last, because bitter substances and especially quinine salts are very liable to adhere to the tongue.

A. The dependence of the anaesthetic action on a number of factors will be presented. These factors are the chemical structure of the anaesthetic and its concentration, the biological substrate, the duration of the applica-tion of the anaesthetic, the time after application, and the subject tasted.

(a) The action of different anaesthetics is quite different. From the data in Table 1 it is apparent that some substances have a very strong, and others a very weak effect on the gustatory field. To the first mentioned group belong Pantocain, Psicain *new*, Subcutin and Anaestheform, and to the latter mainly Tutocain and Novocain. This is most easily seen from the figures in the column labelled *bitter*, *test subject I*. Even 10 min after the application of Pantocain the threshold is many thousand times higher than normal while no threshold value at all could be obtained 10 min after Psicain *new*. Even application of powdered quinine hydrochloride to the tongue gave no taste sensation at all. It only felt like a sandy powder. Tutocain on the other hand showed only a 33-fold threshold increase after 1 min, and Novocain a 22-fold increase.

The findings on the increase in threshold are on the whole duplicated in the other three substrates (salty, sour and sweet), but to a lesser degree. According to their susceptibility to most anaesthetics, the four substrates may be arranged in a sequence :

$$\text{bitter} > \text{salty} > \text{sour} > \text{sweet}$$

It should be definitely pointed out, however, that this is *not* the same sequence as is obtained by arranging the substrates according to their physiological sensitivity as measured by their threshold to our four gusta-

tory test substances.   This sequence, according to new investigations is :

<div align="center">bitter > sour > salty > sweet</div>

However, this is correct only if the new, synthetic sweet substances are not taken into account.

TABLE 1. PROPORTIONAL INCREASE OF GUSTATORY THRESHOLDS t MIN AFTER EXPOSURE TO 0.3 M ANAESTHETIC SOLUTIONS.   A DASH — INDICATES THAT NO THRESHOLD COULD BE FOUND DUE TO THE POWERFUL EFFECT OF THE ANAESTHETIC.

Substance	t	Test subject I				Test subject II			
		Bitter	Salty	Sour	Sweet	Bitter	Salty	Sour	Sweet
Anaesthesin	1	55.6	30.0	15.0	15.0	66.7	30.0	20.0	15.0
	5	5.5	5.0	3.0	4.0	5.5	4.0	3.5	3.0
	10	3.3	3.5	1.5	2.5	4.4	3.0	2.5	2.5
Anaesthe-form	1	—	35.0	30.0	40.0	>100.0	40.0	25.0	35.0
	5	6.6	10.0	5.0	10.0	6.6	10.0	4.0	15.0
	10	3.3	4.0	2.5	4.5	4.4	4.0	3.0	5.0
Subcutin	1	—	50.0	35.0	15.0	111.1	50.0	25.0	15.0
	5	22.2	15.0	15.0	5.0	8.8	15.0	10.0	4.5
	10	11.1	10.0	4.5	4.0	4.4	5.0	5.0	4.0
Novocain	1	22.2	3.5	3.0	3.0	22.2	5.0	3.0	4.0
	5	5.6	2.5	2.0	2.0	6.7	3.0	2.0	3.0
	10	3.3	2.0	1.0	1.5	4.5	2.0	2.0	2.5
Tutocain	1	33.3	4.0	3.0	3.5	33.3	5.0	4.0	4.0
	5	5.6	2.0	2.0	2.0	7.8	3.5	2.0	3.0
	10	1.0	2.0	1.5	1.0	3.0	2.5	1.0	2.5
Pantocain	1	—	—	50.0	—	—	—	—	—
	5	—	—	25.0	—	—	—	—	—
	10	4436	—	10.0	50.0	—	—	20.0	—
Alypin	1	66.7	4.5	1.5	4.5	77.8	4.0	3.0	5.0
	5	22.2	4.0	1.0	4.0	22.2	3.5	2.0	4.0
	10	8.9	3.5	—	3.5	10.0	2.5	1.0	3.0
Cocain	1	—	—	20.0	20.0	—	—	15.0	20.0
	5	10.0	35.0	5.0	15.0	22.2	20.2	4.5	10.0
	10	7.8	15.0	3.5	10.0	8.9	10.0	3.5	10.0
Psicain *new*	1	—	—	20.0	35.0	—	—	15.0	25.0
	5	—	50.0	10.0	20.0	111.1	15.0	10.0	10.0
	10	—	20.0	3.5	10.0	22.2	10.0	4.5	5.0
Tropacocain	1	55.6	5.0	4.5	10.0	66.7	5.0	4.5	10.0
	5	6.7	3.0	3.5	4.0	4.4	3.0	3.5	5.0
	10	3.3	2.5	2.5	3.0	2.2	2.5	2.5	4.5

Sometimes there are certain deviations from the former sequence. Cycloform for example, often affects the salty substrate more than the bitter. The sweet substrate is more susceptible to Alypin than the sour substrate. The Tropacocain susceptibility sequence is

bitter > sweet > salty > sour

Subcutin and Anaestheform had a very strong effect on all substrates, taste subject I. The bitter substrate, however, as always the most susceptible. Thus, 1 min after Subcutin no threshold for quinine could be obtained ; the threshold increased 50 times for NaCl, 35 for tartaric acid and 15 for cane sugar.

Also those anaesthetics which were used in a ten times lower concentration because of their low solubility had varying effects on the different substrates. Particularly effective were Acoin and Eucupin (Table 2). There was no quinine threshold to be found 10 min after Acoin application and 1 min after Eucupin the quinine threshold was more than a hundred times increased, and even after 10 min it was about 11 times normal.

TABLE 2. PROPORTIONAL INCREASE OF GUSTATORY THRESHOLDS t MIN AFTER EXPOSURE TO 0.03 M ANAESTHETIC SOLUTIONS. A DASH — INDICATES THAT NO THRESHOLD COULD BE FOUND DUE TO THE POWERFUL EFFECT OF THE ANAESTHETIC.

Substance	t	Test subject I				Test subject II			
		Bitter	Salty	Sour	Sweet	Bitter	Salty	Sour	Sweet
Eucain β	1	10.0	4.0	4.0	3.5	10.0	4.0	4.0	3.5
(0.04 M)	5	2.2	3.0	1.5	2.5	3.3	3.0	2.5	3.0
	10	1.1	2.5	1.0	2.0	2.2	2.5	1.5	2.5
Diocain	1	88.9	20.0	10.0	10.0	55.6	20.0	10.0	5.0
	5	33.3	15.0	4.0	5.0	44.4	10.0	3.5	4.5
	10	22.2	10.0	3.5	5.0	11.1	10.0	3.0	4.5
Acoin	1	—	5.0	2.5	4.5	—	10.0	4.5	4.5
	5	—	4.0	1.5	4.0	—	5.0	3.5	4.0
	10	—	3.0	1.0	3.5	—	4.5	2.5	3.5
Percain	1	44.4	15.0	4.0	10.0	55.6	10.0	4.5	10.0
	5	22.2	10.0	2.0	5.0	22.2	5.0	3.0	5.0
	10	11.1	10.0	1.5	4.5	10.0	5.0	2.5	5.0
Eucupin	1 >	100.0	45.0	—	5.0	100.0	40.0	—	4.0
	5	22.2	10.0	10.0	4.0	22.2	5.0	10.0	3.5
	10	11.1	5.0	4.5	3.5	6.6	3.5	5.0	3.0

The salty substrate is also very susceptible. Eucupin and Diocain had a very marked influence, increasing the 1 min sodium chloride threshold to

45 and 20 times the normal respectively. The effect on the sweet and sour substrates was much less pronounced. It must however be pointed out that 1 min after Eucupin no tartaric acid threshold could be obtained. Nevertheless, there are anaesthetics which markedly influence the sensitivity of all substrates. There are also some anaesthetics which exert a very strong influence on the bitter substrate, while the other three substrates are relatively much less influenced. The decisive factor here seems to be the substance itself, not its concentration. Indicating this is Acoin, which has as much effect on the bitter substrate in a 0.03 M solution as 0.3 M Pantocain, while the effect on the salty substrate is much higher with the latter than the former.

(b) Some experiments have shown that the length of time during which the brushing-on of the anaesthetic is performed has a profound influence on the effect. The longer the tongue is exposed to the anaesthetic, the stronger is the effect. There is, of course, a limit to this, as an equilibrium between supply of anaesthetic by brushing-on and removal through the blood vessels is reached.

(c) Immediately after the application of anaesthetic to the gustatory field, the threshold increases and soon reaches a high level. The decrease of sensitivity thus comes very fast, obviously in connection with a fast entry through the epithelium of the tongue. Then not only the gustatory but also the pressure receptors are affected. The effect on them however is transient only. The original increased threshold decreases rapidly for 5 min and then decreases gradually until the original threshold to the test substances is reached 15 to 80 min later. Of course there are differences in this time course, depending mostly on the substrate but also on the individual and the anaesthetic. Some features of this time course are constant : at first a fast rise, then a fairly fast fall from the first to the fifth minute, and at last a very gradual recovery.

During the investigation it was found, as expected, that there are differences between individuals, which are expressed quantitatively rather than qualitatively. Thus the above presented sequence of susceptibility of the four substrates varies. The susceptibility of the sweet substrate to 0.03 M Pantocain was higher for taste subject 2 than for taste subject 1. The threshold increase measured after 1 min was 30 in the first case, and only 5 in the second. This individual difference in susceptibility is not surprising, because in many investigations it has been proved that the gustatory sense shows large individual differences.

B. None of the chosen anaesthetics had a lingering effect, or a permanently damaging one. Thus, basically it is a completely reversible effect. A discussion is needed of the recovery time, however. The effect of a variation in concentration is rather small. For example the recovery time after 0.03 and 0.3 M Pantocain is 40 to 60 and 60 to 80 min respectively

TABLE 3. DURATION OF THE EFFECT IN MINUTES, TABULATED ACCORDING TO TEST OBJECT AND BASIC SENSATION. AN ITALIC FIGURE INDICATES THAT A PERIOD OF SUPERSENSITIVITY PRECEDES THE COMPLETE RECOVERY. C=MOLAR CONCENTRATION.

Substance	C	Test subject I				Test subject II				Test subject III				Test subject IV			
		Bitter	Salty	Sour	Sweet	Bitter	Salty	Sour	Sweet	Bitter	Salty	Sour	Sweet	Bitter	Salty	Sour	Sweet
Subcutin	0.30	50	50	*45*	35	40	50	*50*	45	50	50	55	40	55	50	45	30
Eucupin	0.03	50	50	40	40	50	50	40	45	50	40	40	50	35	35	40	45
Pantocain	0.30	60	85	50	65	65	85	55	70	60	80	45	75	*60*	70	45	75
Acoin	0.03	40	35	10	25	45	40	25	30	40	30	25	30	45	40	25	30
Alypin	0.30	*45*	50	5	25	*40*	30	10	25	*35*	25	25	20	*40*	35	10	25
Tropacocain	0.30	20	25	15	30	20	25	20	40	15	25	15	30	20	20	15	25

(Table 3). Thus a tenfold increase in concentration increases the recovery time less than two times. On the other hand the recovery times after 0.03 M Acoin and 0.3 M Alypin are both 40 to 45 min while the recovery times after the weaker anaesthetics Anaesthesin and Tropacain are 15 to 30 min. Thus, the recovery time depends more on the anaesthetic than on its concentration.

There are differences however among the four substrates. If one of the substrates is only slightly susceptible to a certain anaesthetic, the recovery time will be short. After application of 0.3 M Subcutin solution recovery times for the bitter and salty substrates are 50 min, sour 45 min and sweet 35 min. However Alypin caused still more pronounced differences in recovery times. Three minutes brushing-on of a 0.3 M solution in test subject I yielded a recovery time for bitter of 45 min, salty 50 min, sour 5 and sweet 25 min. Similar, but not identical figures were obtained for the three taste subjects. The complete recovery of the different substrates never occured simultaneously. Thus one can conclude that the substrates are largely independent, and the recovery time is shorter the less a specific substrate has been affected by the anaesthetic.

In this connection the interesting observation was made that the complete recovery to preanaesthetic conditions was often preceded by a period of hypersensitivity. This condition is particularly dependent on the anaesthetic, the substrate and the individual. That the anaesthetic is of importance is shown by the finding that hypersensitivity occurs after Subcutin, Pantocain and Alypin, but never after Anaesthesin, Larocain, Stovain, Psicain *old* and *new*, Tropacocain, Eucain β, Holocain, Diocain, Acoin, Percain and Eucupin. The bitter substrate often showed hypersensitivity while the other three substrates seldom did. The salty substrate showed hypersensitivity only rarely. The hypersensitivity is to no small extent a matter of the individual. With many persons it was very pronounced, at least for some substrates, while in others it was not found.

C. It was of course desirable to determine whether a correlation exists between the chemical structure of an anaesthetic and its effects on the gustatory substrates.

A strict correlation between the chemical structure and the effect on the gustatory field has not been found. One gets the impression that substances belonging to the amidines and the guanidines are more effective than those belonging to other groups. The most potent were Pantocain, which is a derivative of p-aminobenzoic acid alkylaminester, and Acoin, which is a guanidine derivative. In equal concentrations (0.03 M) Acoin is somewhat more powerful than Pantocain, at least on the bitter substrate.

It might be argued that the differences in susceptibility of the four gustatory substrates could be due to an unequal depth of the receptors beneath the tongue epithelium, as is the case with other sense modalities,

such as pressure, warm, cold and pain. Against this interpretation is the fact that the taste buds are located very superficially in the gustatory area. The different susceptibilities of the four gustatory substrates to anaesthetics are thus determined only by the substrates themselves.

SUMMARY

On the basis of our findings the following can be stated : The efficiency of the gustatory receptors is impaired by anaesthetics usually in varying degrees. The substrate of bitter is most susceptible to anaesthetic. How much the other three substrates are impaired depends mostly on the anaesthetic itself. The effect increases very fast and declines rapidly at first, then slower, until after at most 80 min the tongue field has regained its original state. The effect shows individual variations, both quantitatively and qualitatively.

It can generally be concluded that each principal sensation corresponds to its own substrate. These substrates are fairly independent of each other. This is shown by the following facts : Their recovery to the original sensitivity takes quite different times, and complete recovery is sometimes preceded by a period of hypersensitivity.

DYNAMICS OF TASTE CELLS

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INTRODUCTION

How does a chemical stimulus initiate the response of a taste receptor? Direct observation is not feasible at the present time so more indirect approaches must be used. One of the most quantitative and objective is to measure the electrical correlates of the taste cell response and relate them to the physicochemical properties of the stimulus. Chemical reactions depend upon many properties of the atoms or molecule involved and therefore no single parameter can be expected to define the stimulus of the chemical senses.

The experimenter usually considers the taste cell as very stable and unchanging during the course of his experiments. The biophysicist often refers to it as a transducer. Recent evidence, however, has shown that the taste cell is one of the fastest ageing cells in the animal body, since it may only live for several days (Beidler, 1961a). It is the purpose of this communication to summarize some of the properties of taste cells giving particular attention to possible functional effects due to the high turn-over rate of taste cells.

ANATOMICAL CONSIDERATIONS FOR ELECTROPHYSIOLOGISTS

The taste buds of mammals are associated with the fungiform papillae on the front two-thirds of the tongue and with the foliates and circumvallates on the back of the tongue. Those on the anterior portion of the tongue are more accessible to the experimenter and therefore are much more studied.

The chorda tympani nerves innervate the taste buds of the fungiform papillae. The total chorda tympani nerve is often cut and used by electrophysiologists to study the taste receptor responses. This nerve contains not only taste nerves but also other sensory and motor nerves as shown by Foley (see Table 1). Cutting the nerve prior to electrical recording may interrupt efferent pathways to the tongue. The importance of sympathetic activity on taste responses of the rat was studied by Kimura and found to be present but small (Kimura, personal communication).

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The number of taste buds varies with the species as is shown for the circumvallate papillae in Table 2. Although the number of available sensory receptors is often associated with the ability to discriminate quality

TABLE 1. CHORDA TYMPANI OF CAT.

Total axons	1955
Motor Axons	798
Myel.	639
Unmyel.	159
Sensory axons	1157
Myel.	949
Unmyel.	208
Total taste buds	575

From Foley, 1945.

TABLE 2

Animal	Number Taste buds in circumvallate papillae
Chipmunk	750
Hare	1200
Rhesus monkey	1800
Opossum	2900
Wombat	3500
Dog	8000
Pronghorn antelope	48,000

From Tuckerman, 1890–1

in other sensory systems, this is of less importance in taste. In some cases a human can discriminate sour, salty bitter and sweet, when the sapid substances are limited to but one fungiform papilla containing only a few taste buds (Öhrwall, 1891). The number of activated taste nerves is not known precisely in this case, but can be estimated as between 6 and 12.

The structure of the taste bud was often described around the turn of the century. Recently the electron microscopists have reinvestigated the taste buds and the most recent findings have been described by de Lorenzo in an earlier chapter of this book. It is important for the electrophysiologist to note that a single axon in the chorda tympani nerve innervates many (possibly 4–8) different taste cells of a single taste bud and that one taste cell may be doubly innervated. Thus the receptor-axon relationship is not

a simple one in the taste system. The possibility of other interactions between taste cells in the taste bud is given by the fact that de Lorenzo notes many fine bridges between adjacent taste cells.

When taste buds were first discovered, the cells within were classified into two groups and thought to be supporting and sensory in function. Later anatomists examined the cells more closely and classified them into many more types. Still others noted signs of degeneration within the taste buds and also mitotic structures which led them to suggest that all the cells within the taste bud were taste cells but in different stages of development (Parker, 1922). It was reasoned several years ago that if the taste cells were being continually replaced, this could be demonstrated by injecting the rat with colchicine, a known mitotic inhibitor (Beidler, 1961b). This was done and histological examination showed mitotic division in the germinal epithelial cells of the surface of the tongue as well as around the taste bud, but never inside the taste bud. Thus, mature taste cells do not undergo mitotic division. However, electrophysiological recordings show a marked drop in magnitude of response to chemical stimuli as early as 2–3 hr after colchicine injection and no response after 8–10 hr when histological investigation shows the taste buds to be degenerating. Does this mean that colchicine blocks cells surrounding the taste bud from dividing and moving into the taste bud to form taste cells, or is colchicine merely eliciting a very toxic reaction? The chromosomes of newly dividing cells can be tagged by labelling a nucleic acid precursor, thymidine, with an isotope. This was done in the rat and the fungiform papillae examined by autoradiographs to reveal the epithelial cells around the taste bud dividing and some of the daughter cells entering the taste bud (see Fig. 1). Quantitative data reveal an average life span of less than 3–5 days for the rat taste cells. Recently de Lorenzo repeated the above-mentioned experiments using the foliate papillae of the rabbit and confirmed the concept of continuous taste cell replacement (de Lorenzo, personal communication).

No longer can the researcher consider the taste receptors as merely transducers without adequate consideration of their biological character. The duration of a single electrophysiological experiment can be an appreciable fraction of the life span of the cell. Also the histochemist must consider the fact that the array of biochemicals in the taste bud are different than would be the case if the taste cells were of a more permanent character and showed neither modulation nor degeneration.

The continual formation of new taste cells may be related to the fact that taste buds degenerate and disappear shortly after the taste nerve is cut. We placed alcohol on the rat chorda tympani in the middle ear, and within 4–8 hr the taste buds became smaller and the epithelial cells around the taste buds thickened. After 96 hr only a few taste cells remained, and no taste pore was present. In 7 days there were no signs of taste buds. As

the taste nerve regenerates and reinnervates the papillae, the taste buds reform. Similar experiments have already been undertaken by Sandmeyer (Sandmeyer, 1895); and Boeke (1917) and Guth (1958) substituted a non-taste sensory nerve, and taste buds also reformed. How the sensory nerve acts to modulate the taste-cell-forming epithelial cells is not known.
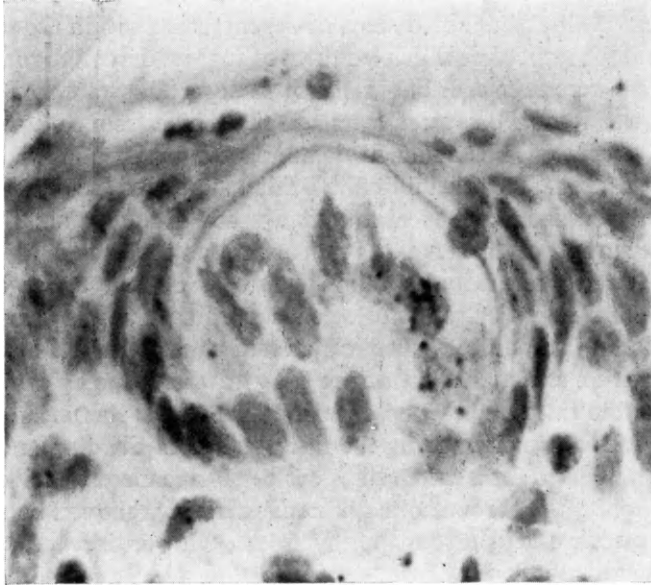
FIG. 1. Autoradiogram of rat fungiform papilla taste bud 200 hr after intra-peritoneal injection of tritiated thymidine; ×1000. Notice labelled cells within the taste bud. (From Beidler, 1961a.)

What happens to the neural innervation of a single taste cell as it ages and moves toward the center of the taste buds? This is not known. However, it is very plausible that the nearest nerve fiber branches to innervate the newly forming taste cell at the outer rim of the taste bud. The taste cell moves toward the center of the taste bud at the slow rate of about 0.06μ/hr (10Å/min or 1.4μ/day). In this manner the taste fiber at the rim of the taste bud would always innervate young taste cells in contrast to the fiber at the center of the bud which would innervate the older cells. By this process the specificity of single taste nerve fibers would be maintained even though the individual receptor cells could continually age and change in their response characteristics.

CHARACTERISTICS OF TASTE CELL MEMBRANE

Electron microscopy reveals that the cells within the taste bud are very tightly packed so that there is little probability that taste stimuli applied to

the surface of the tongue enter between the cells of the taste bud. This is in agreement with many physiological experiments that indicate difficulty in penetration of substances through the tongue surface. For example, mitotic division is blocked within 15 min after colchicine is injected intra-peritoneally into the rat although no blockage is seen 24 hr after much higher concentrations of colchicine remain on the tongue surface. The microvilli of the taste cells, on the other hand, extend into the taste pore and are in direct contact with the saliva. For the above reasons it is assumed that the microvilli are the structures that are stimulated by taste solutions.

A single thickness membrane of about 80Å encloses the contents of each microvillus as far down as the crypts where the membrane thickness doubles (de Lorenzo, 1962). The gross chemical constituency of this membrane is not known, but it is probably similar to that of other cell membranes ; namely oriented protein and phospholipid. Direct chemical or physical analysis of the membrane is not presently possible but the chemical stimulus can be used as an ionic or molecular probe of the surface of the microvilli. This was attempted in 1951 by setting up a theoretical approach to the binding of the stimulus to the receptor, showing its agreement with experimental data, and then calculating the binding forces involved (Beidler, 1954).

The ionic or molecular stimulus, A, can be assumed to be adsorbed to sites on the microvillus surface, B, to form AB. The equilibrium constant, K, for such a reaction is

$$K = \frac{[AB]}{[A]\,[B]}$$

It is assumed that the magnitude of taste receptor response, R, is proportional to the number of sites filled, AB, and that the maximum possible response, R_s, occurs when all the available sites are filled. From such reasoning, the equation

$$\frac{C}{R} = \frac{C}{R_s} + \frac{1}{KR_s}$$

is found where C is the concentration of the stimulus. This taste equation was found to satisfy much of the experimental data and a distinct value for K, which determines the affinity of the taste receptor site for the taste substance, was obtained for each type of stimulus (Beidler, 1954).

The magnitude of maximum response, R_s, can also be determined and is a measure of both the number of total receptor sites available for the particular stimulus and the intrinsic activity of each occupied site. The intrinsic activity is a function of the ability of the stimulus-receptor combination to initiate a response, presumably by producing a small local

change in the configuration of a macromolecule on the receptor surface and forming a hole which allows potassium ions to leak out of the receptor. Since thermodynamics show that $\Delta F = - RT \ln K$, the change in free energy, ΔF, which is a measure of the binding force, can be readily obtained. The magnitude of these forces, several kilocalories per mole, is in agreement with the concept that the chemical stimulus is adsorbed to the receptor and that enzymatic reactions are not involved in the initial response-triggering process, although, of course, they are important in metabolic reactions that produce the energy necessary for the electrical taste cell response.

Many different types of ions and molecules can interact with taste receptors and stereoselectivity has been shown (Steinhardt, 1962). This implies different types of receptor sites, some showing complementariness. Van de Waals forces, hydrogen bonding, dipole-dipole interactions, ionic forces, etc., are important in the stimulus-receptor binding and no single parameter can be expected to exist which would be a direct measure of stimulating efficiency of a taste substance. Not only must the reactive groups of the taste substance be considered, but also their spatial arrangement and degree of hydration.

One type of receptor site may be filled by several kinds of ions or molecules with both different binding energies and different efficiencies in response elicitation (intrinsic activities). Their competition for the same site can be described mathematically (Beidler, 1961a). Several different sites that react to two or more independent kinds of stimuli may exist on one receptor surface. One such example is the existence of sites for Na^+ on the same receptor surface containing sites for fructose or other sugars.

The types of receptor sites and the number of each vary from receptor to receptor and from one species of animal to another. It is possible for a given chemical stimulus to react with two different types of receptor sites. In this case two equilibrium constants are necessary to describe the experimental data. One example is NH_4Cl (Beidler, 1961a). Mixed tastes are often associated with such stimuli.

The membrane of the taste receptor is electrically charged. A micropipette thrust into a taste receptor shows that such a charge separation exists across the membrane and that it decreases with chemical stimulation of the receptors (Kimura and Beidler, 1961). It is likely that the potential difference is due to an ionic gradient similar to that which exists in other sensory cells. However, it must be remembered that the microvilli do not live in a medium of fixed chemical ingredients. Saliva normally surrounds the microvilli but is often replaced by the many taste substances taken into the oral cavity. It is also known that water can be continually flushed over the surface of the tongue for 24 hr with little effect on the

subsequent magnitude of response to suprathreshold chemical stimuli. Continuous adaptation to distilled water can, however, lower the salt threshold for human subjects (Pfaffmann and McBurney, 1962).

RESPONSE OF TASTE CELLS, NERVE FIBERS AND NERVE BUNDLES

Single Taste Cells

The magnitude of electrical depolarization of the taste cell, as measured with the aid of a KCl micropipette, is taken as an index of the response of the taste cell to applied chemical stimuli. The resulting stimulus-response curves can be satisfactorily described by the fundamental taste equation. Each taste cell, however, differs in overall sensitivity as well as its relative magnitude of response to a series of select stimuli such as various concentrations of NaCl, sucrose, HCl and quinine HCl (see Table 3). Many taste cells do not respond to sucrose, but others do. Some respond to sucrose as well as to salt and acid. Thus, highly specific taste receptors that respond to but one type of stimulus are not found (Kimura and Beidler, 1961).

TABLE 3. RESPONSE IN MILLIVOLTS OF THE HAMSTER TASTE RECEPTOR TO BASIC STIMULI OF FOUR TASTE QUALITIES

Taste receptor	0.1 M NaCl	0.1 M sucrose	0.02 M quinine	0.01 M HCl
1	10.5	2	4.5	—
2	8.5	2	7.5	0
3	9	1	4.5	—
4	11.5	5	8	22.5
5	10	6.5	9	12.5
6	6.5	1.5	5	—
7	9.5	2.5	7	9
8	14	0	5	14

From Kimura and Beidler, 1961.

Single Chorda Tympani Taste Fibers

Since a single nerve fiber may innervate several taste cells, how specific are the single fibers ? The average frequency of nerve impulses per unit time is taken as an index of the magnitude of response of the unit (all the cells innervated by the single taste fiber) to applied chemical stimuli. The researches in the laboratories of Zotterman (Cohen, Hagiwara and Zotterman, 1955 ; Zotterman, 1955), Pfaffmann (Pfaffmann, 1941 ; 1955) Sato (Sato and Kusano, 1960) and the author's (1957) have all shown

that highly specific taste fibers are very few in number (see Fig. 2). In fact, the results are similar to those obtained by recording single taste cells. How information concerning taste quality is relayed to the higher nervous centers will not be considered here since both Pfaffmann, (1959, 1960) and Zotterman (1955 ; 1959) have studied this problem for many years and published elsewhere.
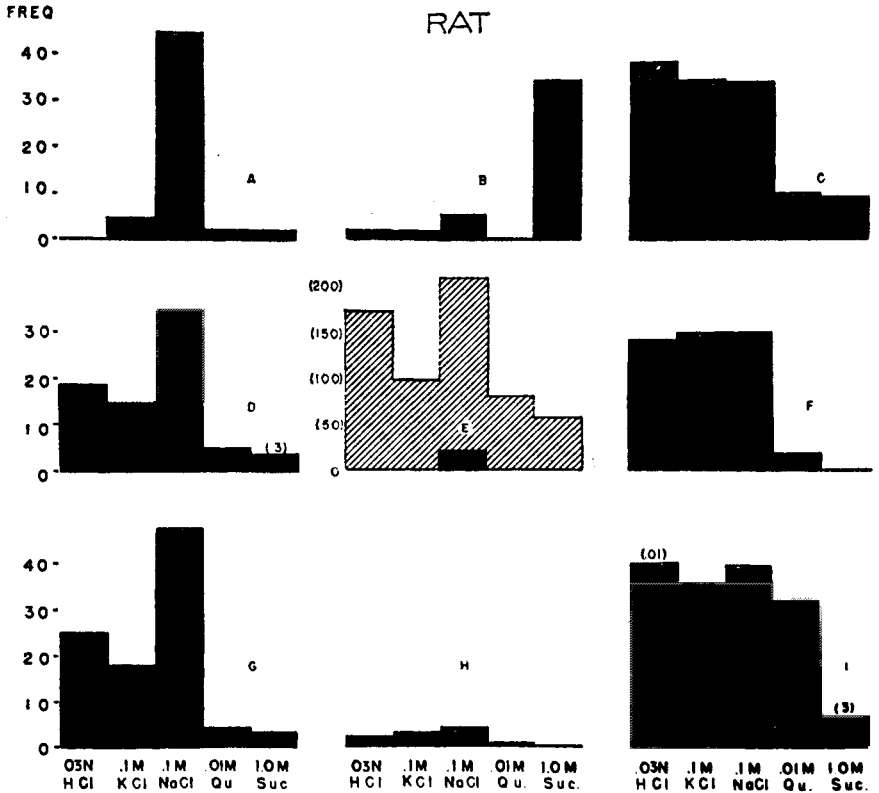
FIG. 2.   Histograms summarizing frequency of neural response during first second to five taste solutions in nine single chorda tympani nerve fiber pre- parations in rat.   Cross-hatched histogram superimposed on histogram for single fiber E shows relative magnitude of summated response (indicated by arbitrary units in parentheses) for same taste solutions.   (From Pfaffmann, 1955.)

It should be remembered that the single taste fiber may reflect informa- tion coming from sources other than taste cells ; namely, bare nerve endings near the taste pore but between taste cells. This is particularly true of responses to salts where discrepancies between response of single taste cells and single taste fibers have been noted (Kimura and Beidler, 1961).

What function can bare nerve endings play in responses to chemicals and how specific are the responses to various stimuli ? This can be best

determined experimentally by recording from a small trigeminal nerve bundle that innervates the olfactory area. These small sensory fibers end in the mucosa as bare nerve endings and their very low firing rate allows the experimenter to analyze in a simultaneous recording as many as 7 single fibers that respond to an applied vapor. Such simultaneous recordings indicate that the bare nerve endings are highly sensitive to chemical stimuli and that certain fibers respond best to one type of chemical whereas others respond better to another type. Thus one might expect that bare nerve endings in other tissue also respond well to chemical stimuli and even show some specificity in their response to a number of different types of chemicals.

Chorda Tympani Nerve Bundles

The response of a large population of taste buds may be studied by recording from the total chorda tympani nerve bundle. The frequency of nerve impulses from single taste nerve fibers is very irregular and therefore difficult to quantitate. For this reason an integration of the total recorded activity from the chorda tympani nerve bundle is used for many studies (Beidler, 1951). Fishman showed that the results of such integration resembles that of the sum of a number of single fibers (1957). Since the total area under the curve of the recorded action potential is integrated, and since the smaller fibers have a smaller height but a greater width, the disparity in contribution between large and small fibers is not as great as one might expect. Integration is not, however, a useful technique for the study of the physiological basis of taste qualities.

RESPONSE CHARACTERIZATION

Temporal Characteristics

Following stimulation the frequency of nerve firing at first increases and then declines rapidly during the first second or two and reaches either a constant steady state, as in the case for the response to NaCl in a good and fresh rat preparation, or a slowly declining state. This initial increment is not as stable and reproducible as the steady state and is probably a function of the properties of the nerve fiber rather than the taste cell since it is not present in single taste cell recordings. The initial transient response is not linearly related to the magnitude of the steady state response that may follow, but it may be very important at low stimuli concentrations. The rat threshold to NaCl may be as much as a hundred or thousand times lower when the intial response rather than that of the steady state, is used as a criterion of response. It should be noted that when the electrical neural activity is integrated, the magnitude of the

initial transient response is determined by the time constant of the elec-
tronic integrator and thus varies in published results from one laboratory
to another.

——=20 Sec.
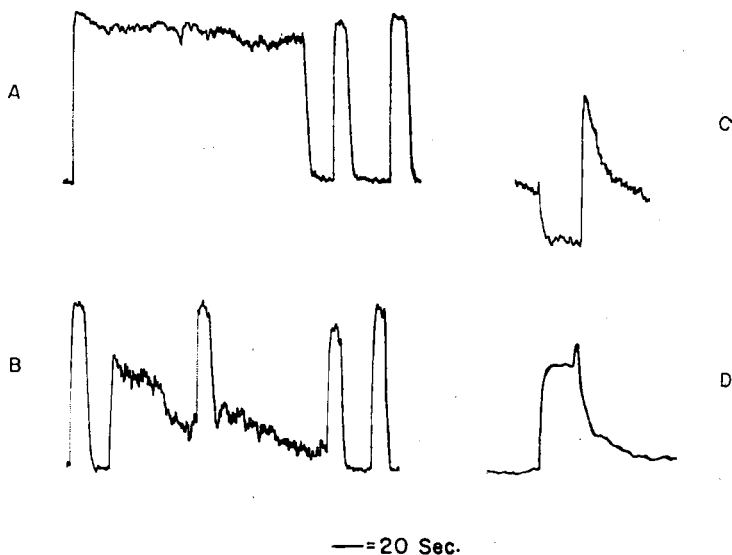
FIG. 3. (A) Continuous response to 0.1 M NaCl followed by water rinses and
two additional responses to 0.1 M NaCl. (B) Response to 0.1 M NaCl followed
by continuous response to 0.1 M $CaCl_2$ with two responses to 0.1 M NaCl inter-
posed before rinsing with water. (C) Response to 0.1 M K benzoate. Note
negative response followed by a positive transient response during water rinse.
(D) Response to 0.05 M HCl. Note increased transient response during
water rinse.

Although the temporal pattern of the response to many stimuli is
similar to that to NaCl, differences do exist. The rat response to sucrose
may be slow in starting and show grouped nerve impulses when recording
from single taste fibers (Beidler, 1951 ; Fishman, 1957). The response
to $CaCl_2$ never reaches a steady state but continually declines toward
zero over a period of several minutes (see Fig. 3). High concentrations
of $BaCl_2$ produces a response that is slow in starting, steadily increases,
shows a sharp initial increase when water is applied and only decreases
several minutes later. The response to some acids also shows an additional
transient increase when the tongue is rinsed with water followed by a rapid
decrease. Low concentrations of potassium benzoate, on the other hand,
depress any resting activity of the rat, but produce a large transient positive
response when water is again applied to the tongue. Some of these fast
transient responses may be of particular importance to the animal during
normal eating since the concentration of the stimulus applied to any given
taste bud varies continually as the animal moves the food about the

tongue, and no steady state of stimulus concentration is available to the receptor.

Temperature Dependency

Since adsorption is little influenced by small temperature changes, little change in magnitude of taste response to a given substance at various temperatures is expected. This is consistent with experimental results with the rat if the taste stimulus is applied quickly and the response measured. If, however, the temperature change is maintained over a period of a minute or longer, then the metabolism of the taste bud changes as well as the local blood supply and the magnitude of taste response is expected to be very temperature dependent. This is particularly true for cold blooded animals or those taste preparations where the blood supply is not intact and where the whole tongue quickly reaches equilibrium with the temperature of any solution applied to its surface. The difficulties in electrophysiological taste experiments where the temperature varies are compounded by the fact that the chorda tympani nerve bundle contains some temperature fibers. Also, the taste nerves are stimulated directly at temperatures of 7°C or below (Fishman, 1957).

Species Dependency

The first definitive taste experiments on different species using electrophysiological techniques were undertaken independently by Pfaffmann (1955) and Beidler (Beidler *et al.*, 1955). They showed large differences in relative responses to sweet, bitter and sour stimuli as well as to various salts. The hamster responds better to sugars, than does the rat, and the cat responds very poorly. The rat responds well to NaCl compared to KCl whereas the reverse is the case for the cat. An abundance of additional evidence on many other species has been accumulated by other laboratories. It is interesting to note that although most rat taste fibers respond best to NaCl and not as well to KCl, there are some single fibers that respond to KCl better than to NaCl. This suggests that differences in overall taste response from one species to another represents merely a different distribution in the number of receptors responding best to any one chemical stimulus.

Stability of Response

If the taste cells are quick to age and if this results in changes in functional characteristics of the individual taste cells, how stable are the fiber types? No change would be expected since one fiber represents taste cells of the same age group. Since the taste buds degenerate upon cutting the nerve supply, would this change the fiber type? No direct evidence is available but some observations suggest such changes. For example, the magnitude

of sugar response often declines with the duration of the experiment and this decline always precedes that of the decline in the salt response. In particularly long preparations, those over 20 hr, the response to NaCl may decline until the response to KCl is larger. What effect small changes in the blood supply over long periods has on taste responses is not known. The response does begin to decline within 15–20 min after the blood supply is completely shut off to the tongue. The turnover rate of taste cells should be affected by vitamin deficiencies, ionizing radiation, mitotic inhibitors, hibernation, etc., which in turn should be reflected in changes in taste responses. It is known from work in our laboratory that urethane, an anaesthetic as well as a mitotic inhibitor, produces histological changes in the taste bud within 40 hr after intraperitoneal injection.

CONCLUSION

The taste receptors are very sensitive and yet very rugged. They are renewed continually and thus have a short life span. Their response to chemical stimuli varies from one receptor to the next in the same population. The response of the total population is very similar from one individual to the next although large differences are noted from one species to the next. It is not yet known with accuracy how much the response characteristics of a particular single fiber depend upon the experimenter's disturbance of the natural conditions of the taste system nor how they may change with periods of time comparable to a sizeable fraction of the total life span of the individual receptors.

ACKNOWLEDGEMENTS

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DISCUSSION

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DR. BEIDLER has told us that the receptor cells of the taste buds are constantly formed at the periphery of the buds and move inward toward the center of each bud as they mature. The rate of replacement is rapid and the average life of a receptor cell is only a few days. Obviously one or more nerve fibers must make contact with each new cell in turn. Dr. Beidler further suggested, in response to my question, that the nerve fibers do not migrate inward with the receptor cells, but that the receptor cells are passed along, so to speak, from one set of nerve fibers to another.

This situation raises a fundamental problem of neural organization because we know that the pattern of chemical sensitivity varies from one cell to another. Different patterns of response to salt, sweet, bitter and acid substances are demonstrated for different neurons and also, by their receptor potentials, for different receptor cells. But if the connections between receptor cells and neurons are continually changing the patterns of central activity should become disorganized. A predominantly " sweet " fiber would become " bitter ", " bitter" might become " salt", and so on.

Only two escapes from this dilemma appear possible. One is that the afferent neurons continually change their central connections to match the sensitivity of their receptor cells. This possibility I dismiss as fantastic. The other possibility is that the pattern of sensitivity of each receptor cell is determined by the neuron or set of neurons by which it happens to be innervated. This possibility is in general accord with accepted principles of morphogenesis. I understand that Dr. Beidler shares this interpretation with me.

But now let us pursue the implications of this important hypothesis, which is new, as far as I am aware, in relation to the gustatory system. It implies that the specific sensitivity of a receptor cell can be changed and changed rapidly as synaptic contact is made with a new fiber or fibers or as old contacts are lost. There must be a continual flow of " information " in the form of chemical material, very specific chemical material, outward from the cell body of the afferent neuron. This information establishes the code according to which the receptor cell will excite the neuron in response to a particular class of chemical stimuli applied to the taste bud.

Now we also know that each receptor cell and consequently its neuron(s) respond to not only one class of chemical substance but to at least three or four, to different degrees. The variety of patterns of sensitivity is actually so great that it suggests a continuance in each of several dimensions as if these were a random or partially random distribution of sensitivity to each class of stimuli independently of one another.

The type of chemical information that is transmitted outward by each afferent neuron is presumably relatively constant for that neuron. Each neuron may, perhaps, impose only a single class of sensitivity on a receptor cell. This is not a necessary assumption, however. A neuron might transmit more than one chemical sensitizer. The essential assumption is that the character and quantity of the outflowing chemical information are determined by the central connections of the peripheral sensory neuron. We assume that the amount of chemical sensitizer of each class is proportional to the richness of the central connections of a neuron to the ultimate " center " for that class of gustatory sensation. Note that this is almost exactly the same assumption that we have already made for the receptor cells. It is not a new major assumption.

Under these assumptions the response pattern of a particular neuron does not necessarily correspond to the pattern of chemical sensitivity that it transmits to the receptor cell. The receptor cell may receive sensitizers from more than one neuron and we cannot assume that they will be the same. The pattern of centrifugal information is determined by the central connections of each neuron while its response pattern is determined by the complete pattern of sensitivity of the receptor cell.

The differences between the efferent and the afferent patterns brought about by overlapping innervation represent false information or " noise " in the afferent message to the central nervous system. We can assume that this noise is largely eliminated by the familiar principle of mutual inhibition which enhances contrast in the auditory, visual, cutaneous and muscular systems. If only there is some initial bias in the central connections of each sensory neuron, such as might well arise from its spatial relations to the ultimate gustatory centers, and if these biases are different for different neurons, there will be corresponding, although somewhat reduced, biases in the patterns of sensitivity of the receptor cells.

We have assumed that the intensity of the efferent chemical message is proportional to the richness of innervation, i.e. the number of synaptic contacts, both centrally and peripherally. The probability of excitation of nerve impulses also obviously depends on the richness of innervation, according to the principle of summation of electrotonic effects. The distribution of afferent impulses among the sensory neurons is therefore automatically biased in exactly the right way to excite preferentially the center that corresponds to the particular class of chemical compound that happens to be the external stimulus at the moment. The situation is clearest near threshold when only the receptor cells that are richly supplied with the proper chemical sensitizer will develop significant receptor potentials and only the neurons that have supplied that sensitizer richly will have enough synaptic contact with those receptor cells to be excited. If the stimulation is stronger, another neuron that may be biased toward another quality will also be excited by the same receptor cell, but it will discharge at a lower rate. But these " unwanted " impulses are made ineffective centrally by the inhibition from the " right " center that is more strongly activated.

I believe that this model of the organization of the gustatory system deserves attention because it makes no assumptions that do not have close counterparts elsewhere in neurobiology and because it explains simultaneously two puzzling features of gustatory physiology. The first puzzle is the maintenance of neural organization in the presence of rapid turnover of receptor cells in the taste buds. The second is the decoding of the complex variety of patterns of response that are found in the single afferent fibers.

The four major assumptions that are involved are :

(1) The distribution of central and peripheral connections of the afferent neurons is quasi-random but is partially biased.

(2) The pattern of chemical sensitivity of a receptor cell is imposed and maintained by chemical " sensitizers " that are derived originally from several gustatory sub-centers and are delivered to the receptor cells in quantities that correspond to the richness of synaptic contacts at each level.

(3) The degree of stimulation of a receptor by a particular chemical depends on the relative richness of that receptor in appropriate sensitive sites. Initiation of nerve impulses depends also on the richness of synaptic contacts.

(4) Unwanted neural activity is rejected by mutual inhibitory action among the several higher-order sub-centers that determine the specific chemical sensitivities of the gustatory system. Activity in such a sub-center is a necessary condition for sensation of a particular quality of taste and/or discriminatory behavior in respect to a particular class of chemical stimuli.

An interesting feature of this model is its statistical character which makes the overall action independent of the details of the connections of individual afferent neurons or of the exact patterns of sensitivity found for individual neurons.

THE SIGNIFICANCE OF THE TERMINAL STRUCTURE OF AFFERENT NERVE FIBRES

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SPECIALIZED structures, 100 to 300μ in diameter in the superficial layers of hairy skin in the cat, contain in their modified epithelium a layer of large cells, numbering 20 or more, each of which encloses a disc of nervous tissue, approximately 10μ wide and 1μ thick, densely packed with mito-chondria, which is the terminal expansion of a branch of a myelinated afferent nerve fibre (Iggo and Muir, 1962). A discharge of afferent impulses in the myelinated fibre is most easily aroused by mechanical stimulation of the epidermis, threshold 3 to 5 mg, adapts slowly to steady pressure and can also be provoked by a fall in cutaneous temperature (Iggo, 1962). These properties, particularly the rate of adaptation, distinguish the axons from other similar myelinated axons innervating hair follicles in the same skin, and are always associated with the specialized region of dermis and epid-ermis, for which the name " touch corpuscle " is suggested.

If the skin was denervated by cutting the saphenous nerve the distinctive structure of the epidermis was disorganized, the layer of special cells dis-appeared and the epidermis became similar to the adjacent unspecialized parts, but the capillaries and Schwann cells remained in the dermis, at least for 30 days. When, after crushing the saphenous nerve, the afferent fibres were allowed to regenerate, the growing axonal tips did not reveal distinctive responses to mechanical and thermal stimuli, even when the growing axons had reached the dermis (Brown and Iggo, 1962). There was a brief discharge in all axons, with a relatively high threshold, in response to mechanical stimuli and no discharge in response to temperature changes. Two spatial patterns of mechanical sensitivity emerged among the large myelinated axons ; in one type mechanical stimuli were effective over areas of more than a square centimetre while in the other type the res-ponsive areas were restricted to a few square millimetres, probably corres-ponding to hair follicle and " touch corpuscle " axons respectively.

There was a period after the nerves had reached " touch corpuscles " and were dividing in the dermal papilla when the response to mechanical stimuli was still undifferentiated. At this stage the epidermis, which earlier had shown signs of disorganization, had once more thickened but the

specialized basal cell layer was still absent. A few days after this the typical, low threshold, slowly-adapting response to mechanical stimulation reappeared, weakly at first, and with it a response to temperature changes. Coincident with this change was the re-appearance of the specialized epidermal cells, with their enclosed nerve discs and other features.

From these results it can be concluded that the afferent fibres do not possess any selective sensitivity when they are growing into the skin and that in some way the differentiation of the nerves depends on further development which occurs when the axons have reached their terminal sites. For the afferent fibre innervating a " touch corpuscle " this appears to be the formation of nerve discs in association with specialized cells at the base of the epidermis. Whether this type of differentiation or modulation of associated cells by the nerves, or vice versa, occurs with other afferent fibres is not known. The interesting results reported to the Symposium by Dr. Beidler, which establish a progressive change of epithelial cells within the taste bud, suggest that it also occurs there. In general, it can be asked whether the cells associated with the afferent nerve terminals are necessary only for the proper development of their selective sensitivity or whether the selective sensitivity depends more directly on the intervention of the associated cells as transducers.

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THE EFFECT OF TEMPERATURE CHANGE ON THE RESPONSE OF TASTE RECEPTORS

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PREVIOUS experimental results on the effect of temperature change on taste sensation of human subjects are not consistent. It is reported Weber (1847) found that the sensitiveness of taste sensation was greatest when the exciting substance was at the temperature of body (Moncrieff, 1951). However, the work by Hahn (1936) shows that within a temperature range of 17–42°C the sensitivity to sugar increases with an optimum at 37°C, salt and quinine sensitivity decreases and acid is unaffected by temperature.

Recent studies on the effect of temperature on the response of taste receptors of mammals using electrophysiological methods have not yielded significant results. Abbott (1953) found an optimal response at 22°C in rats, Beidler (1954) reported no change in response magnitude of taste receptors in rats for 0.5 M sodium chloride at temperatures of 20°, 25° and 30°C, and Fishman (1957) observed in rats and hamsters smaller response to 0.5–1.0 M salts at 5°C than normally obtained at 25°C. Therefore no systematic investigation on the effect of temperature change on the response from taste receptors covering wide temperature range and various kinds of taste stimuli have yet been accomplished.

Therefore Dr. Nagaki, Mr. Yamashita and I carried out experiments in which response of taste receptors was measured by changing temperature of various kinds of taste solutions. We have adopted two kinds of approaches to this problem. First, the integrated response of the chorda tympani nerve to stimulation of the tongue by various kinds of solutions at varying temperatures was recorded ; second, impulse discharge in a single chorda tympani nerve fibre due to stimulation of the tongue was recorded and analyzed. In order to stimulate a constant area of the tongue, the anterior two-thirds of the tongue were placed in a flow chamber and 100 ml of the solution were flowed over the tongue for 10 to 15 sec, and subsequently the tongue was rinsed with Ringer's solution.

Temperature of the solution and change in the surface temperature of the tongue were recorded with a thermistor. Temperature of the tongue surface of the anaesthetized cat was generally about 30°C and a fairly constant

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linear relationship was observed between temperature of stimulating solutions and change in surface temperature of the tongue. After flowing a solution over the tongue a mean change of 5.4°C in the tongue temperature was produced when there was a difference of 10°C between temperature of the solution and that of the tongue.

For the stimulation we have employed sodium chloride, saccharine sodium, hydrochloric acid, quinine hydrochloride and distilled water, quinine and acid being dissolved in Ringer's solution. Temperature of these solutions was changed from 5°C to 45°C, which resulted in lowering of the tongue temperature to about 15°C and raising it to 40°C. Ringer's solution was also employed for stimulating thermoreceptors only.

As already shown by a number of investigators (Cohen, Hagiwara and Zotterman, 1955 ; Appelberg, 1958), the tongue of cats hardly responds to sweet substances. It was also found in our experiments that the threshold for saccharine was 1/8 M and response to saccharine was nearly the same as that to NaCl. It seemed that chemoreceptors of cats respond to saccharine as sodium salts.

RESPONSE FROM THE WHOLE CHORDA TYMPANI

An example of the integrated response from the whole chorda tympani to Ringer's solution, NaCl and water of varying temperatures is shown in Fig. 1. In general, the response attains its maximum within 1–2 sec and decays thereafter. The time course of decay is slower when temperature is lower. The maximum amplitude of the response was measured and taken as the response magnitude.

As shown in Fig. 1, no response was observed to Ringer's solution at 30°C, while warm or cold Ringer's solution produced a response. Similarly the response to NaCl is small at 30°C, but it is increased with a rise or fall of the temperature. This is more clearly shown in Fig. 2, in which magnitude of the response to various kinds of solutions is plotted against the temperature of solutions. All the responses are minimal at 30°C and are increased with a rise or fall of the temperature.

The response to warm or cold Ringer's solution may result from the response of thermoreceptors. In order to compare the response of the chorda tympani to warming or cooling of the tongue with that of the lingual nerve, which does not mediate taste information, we recorded the response of the lingual nerve when the tongue was stimulated with solutions of varying temperatures. It is easily seen in Fig. 3 that the lingual nerve contains only fibres mediating cold, and it is clear from Fig. 2 that the chorda tympani contains fibres mediating warm and cold sensations. This finding is consistent with earlier findings by Zotterman (1954).

Since it is supposed that the response of the chorda tympani to solutions

of varying temperatures consists of two kinds of responses, one to temperature change of the tongue and the other to taste stimuli, we have carried out further experiments, in which not only temperature but also concentration of taste solutions were changed. The results obtained for NaCl, quinine and HCl are shown in Figs. 4, 5 and 6, and they reveal more
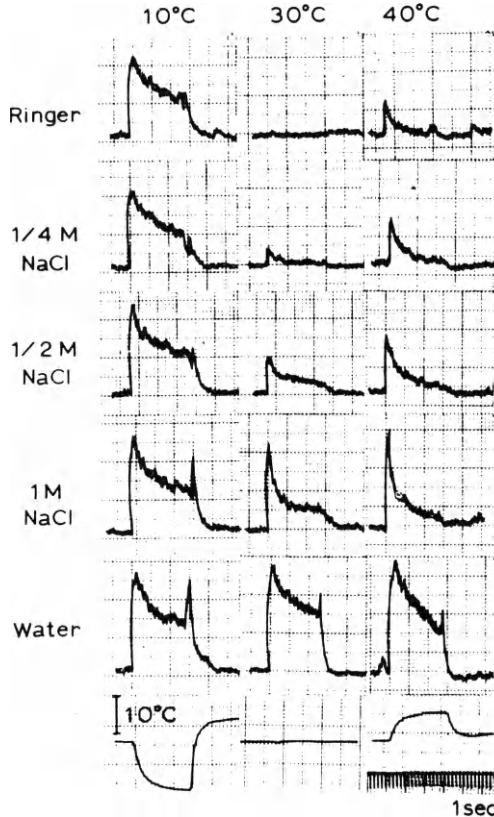
FIG. 1. Integrated response of the chorda tympani to stimulation of the tongue with Ringer's solution, water and NaCl solutions of varying temperatures. Numerals at the top of the figure indicate temperature of solutions, and the bottom records show changes in the surface temperature of the tongue. From Nagaki, Sato and Yamashita, unpublished.

or less similar features. When the concentration is low, the response is minimal at 30°C and is increased with an increase or decrease in the temperature. However, with increasing concentration the response magnitude at 30°C increases more than the increase at the temperature below or above 30°C, and therefore the magnitude of the response to solutions of high concentration does not change very much with a change in temperature. As shown in the righthand figure of Figs. 4, 5 and 6, the increase in the
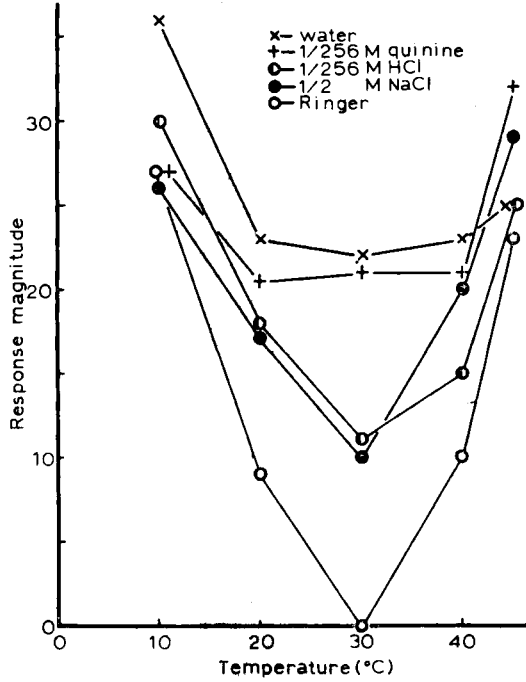
FIG. 2.   Relationship between magnitude of the chorda tympani response and temperature of taste solutions. From Nagaki, Sato and Yamashita, unpublished.
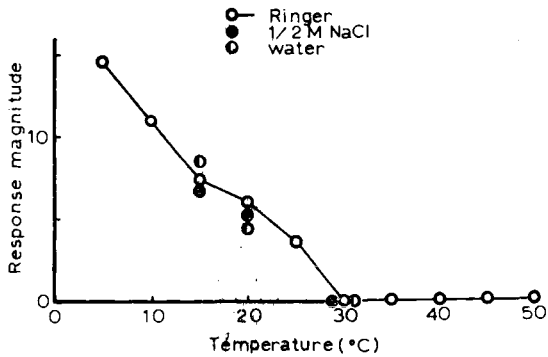
FIG. 3.   Relationship between magnitude of the lingual nerve response and temperature of solutions.   From Nagaki, Sato and Yamashita, unpublished.

response magnitude with an increase in concentration is maximal at 30°C, and consequently it indicates that the taste sensitivity is greatest when temperature of solutions is at 30°C.

FIG. 4. Relationship between the magnitude of the integrated response and temperature of NaCl solutions (A) and that between response magnitude and NaCl concentration at varying temperatures (B and C). Response at arrows indicates the response to Ringer's solution. From Nagaki, Sato and Yamashita, unpublished.

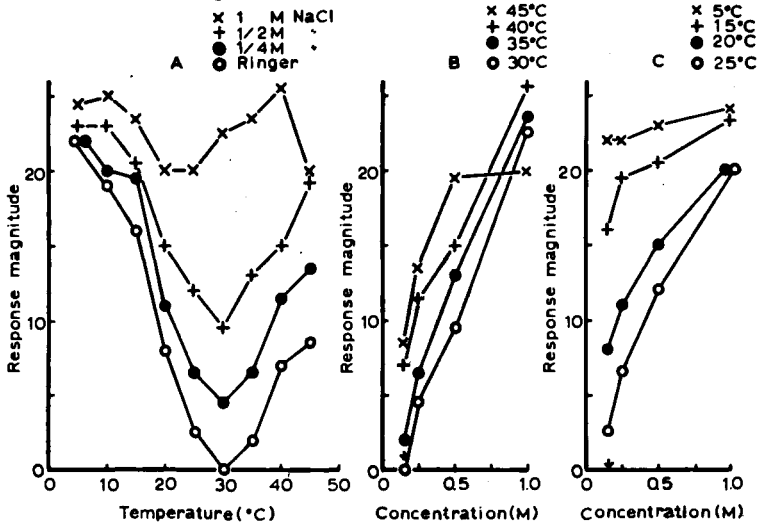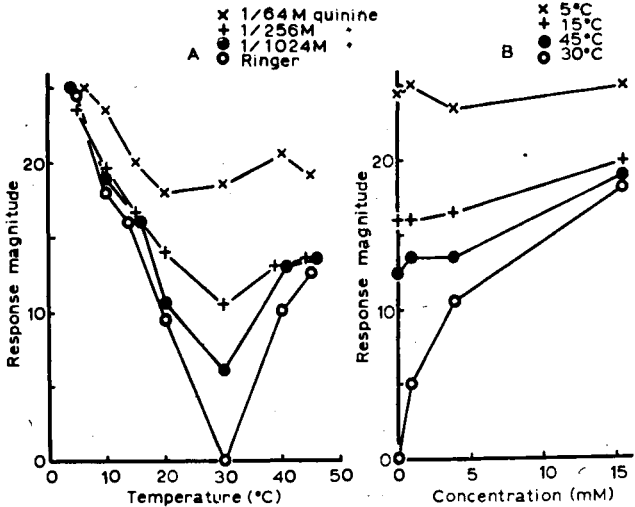FIG. 5. Relationship between the magnitude of the integrated response and temperature of quinine solutions (A) and that between response magnitude and quinine concentration at varying temperatures (B). From Nagaki, Sato and Yamashita, unpublished.

X 1/128 M HCl   X + 6 °C
+ 1/256 M ,   + - 6 °C
● 1/512 M ,   ○ + 4 °C
○ Ringer   ● - 2 °C
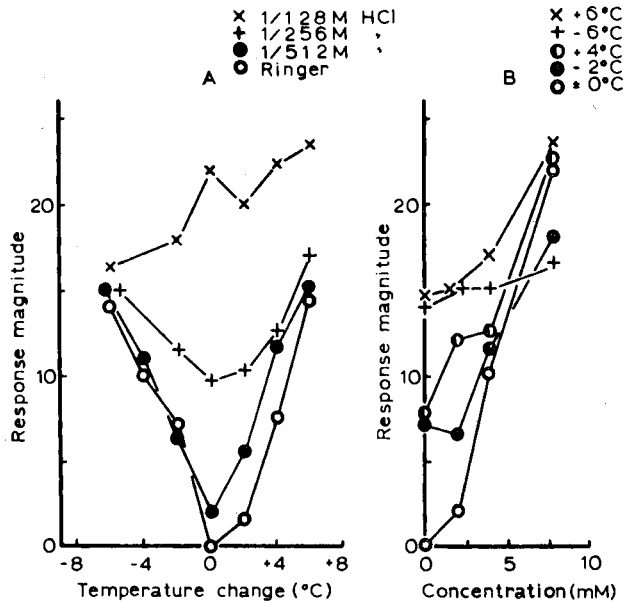   A   B   ○ ± 0 °C

FIG. 6. Relationship between the magnitude of the integrated response and temperature change at the tongue produced by HCl solutions (A) and that between response magnitude and HCl concentration at varying temperatures (B). From Nagaki, Sato and Yamashita, unpublished.

PROPERTIES OF SINGLE TASTE UNITS

Impulse discharge of 29 single nerve fibres was recorded from 24 cats. A majority of single taste fibres of the chorda tympani was found to respond not only to more than two kinds of taste stimuli but also to both taste and temperature stimuli of the tongue. In Table 1 units obtained are classified according to their responsiveness to taste and thermal stimuli. It is seen from this table that about 50 per cent of units obtained responded to warming, cooling and taste stimuli, 30 per cent to cooling and taste, 10 per cent to taste stimuli only and another 10 per cent to temperature change only. In addition, we have also obtained mechanosensitive units, which showed larger spike height than that of other fibres.

Typical examples of the response of four types of units to taste and temperature stimuli are shown in Fig. 7. The unit shown in Fig. 7A responds to warming, cooling and taste stimuli and may be called the *warm-taste unit*. The *warm fibre* in the chorda tympani and described by Dodt and Zotterman (1952) is probably nothing but the warm-taste unit. The unit shown in B responds to cooling and taste stimuli and may be called the *cold-taste unit*. The unit shown in C responds very little to thermal change, but it still shows a small increase in the impulse number when the tempera-

TABLE 1. AFFERENT FIBRES IN THE CHORDA TYMPANI CLASSIFIED
FROM THEIR RESPONSIVENESS TO TASTE AND TEMPERATURE STIMULI
TO THE TONGUE (NAGAKI, SATO AND YAMASHITA, UNPUBLISHED).

Types of units	Number	
A. Units responding to taste, warming and cooling	14	(5)
B. Units responding to taste and cooling	10	(4)
C. Units responding to taste stimuli only	2	
D. Units responding to temperature change only	3	
Total	29	(9)

Numbers in the brackets indicate those of units, which responded
to thermal stimuli intensely. Twenty-four cats were used for this
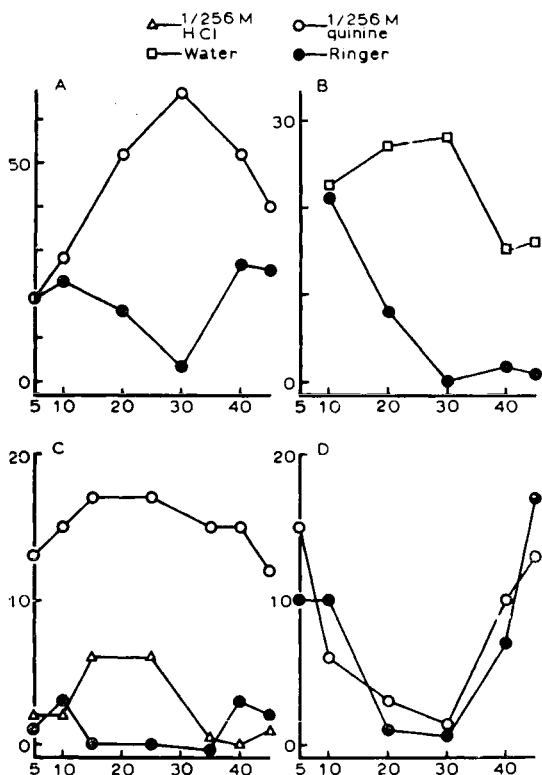experiment.

FIG. 7. Impulse frequency–temperature relationships, obtained with four
different kinds of units. Ordinate ; number of impulses in the first second,
abscissa ; temperature of solutions. From Nagaki, Sato and Yamashita,
unpublished.

ture is 10° or 40°C. The unit shown in D responds to temperature change only. However, it may be possible that this unit responds to taste solutions, if we had employed NaCl, quinine or HCl of higher concentration as stimuli than that employed for testing this unit. Furthermore the frequency of impulses shown in this unit is not greater than that shown in A and therefore the unit shown in D cannot be considered the unit specifically sensitive to temperature change. These results suggest that there are few units in the chorda tympani which are exclusively sensitive to taste stimuli or to temperature change.

Responsiveness of units to 1/2 M NaCl, quinine, 1/256 M HCl and water is shown in Table 2. Results shown in this table agree with those by Cohen *et al.* (1955). Units sensitive to NaCl, quinine or water are relatively

TABLE 2. AFFERENT FIBRES IN THE CHORDA TYMPANI CLASSIFIED FROM THEIR RESPONSIVE-NESS TO FOUR KINDS OF TASTE STIMULI (NAGAKI, SATO AND YAMASHITA, UNPUBLISHED).

Stimuli/ Sensitivity	Number of units					
	NaCl	Quinine	HCl	H₂O	Cooling	Warming
NaCl-sensitive units : 7						
++	7	1	0	0	1	1
+	—	2	5	0	6	4
±	—	1	2	1	0	0
—	—	3	0	6	0	2
Quinine-sensitive units : 8						
++	1	8	0	2	2	1
+	1	—	1	0	4	4
±	1	—	2	0	0	1
—	5	—	5	6	2	2
HCl-sensitive units : 11						
++	5	1	1	2	4	1
+	0	4	10	0	6	3
±	0	2	—	2	0	0
—	6	4	—	7	1	7
Water-sensitive units : 7						
++	0	2	0	7	4	0
+	0	2	2	—	3	4
±	0	3	2	—	0	0
—	7	0	3	—	0	3

Sensitivity + +: indicates initial impulse frequency of more than 15–20/sec to 1/2 M NaCl. 1/256 M quinine, 1/256 M HCl and water, +: 5 — 15/sec, ±: 1 — 5/sec and —: 0/sec at 30°C.

specific, but the unit responding to HCl is also sensitive to other kinds of stimuli and to cooling rather than warming. The unit sensitive to water is greater in spike height than the unit sensitive to NaCl. Difference in properties in large and small fibres is shown in Table 3. The large fibre did not respond to NaCl, while the small fibre did not show response to water. The latter also responded to saccharine, but not the former.

TABLE 3. DIFFERENCE IN RESPONSE BETWEEN LARGE AND SMALL FIBRES, AS MEASURED BY THE IMPULSE NUMBER IN THE FIRST 1 SEC AFTER STIMULI (30°C) (FROM NAGAKI, SATO AND YAMA-SHITA, UNPUBLISHED).

Stimuli	Large fibre	Small fibre
1/2 M NaCl	0	14
1/256 M quinine	10	15
1/64 M quinine	22	1
1/256 M HCl	10	6
1/128 M HCl	37	10
Water	28	0

Response of a cold-taste unit to water and Ringer's solution of varying temperatures is shown in Fig. 8. The unit responded intensely to water and to Ringer's solution of low temperature. The impulse frequency reaches maximum within 1–2 sec after application of the solution and declines thereafter. In general rate of decline in the impulse frequency becomes prolonged with a fall in temperature. This is more clearly shown in Fig. 9, in which impulse number in 1 sec is plotted against time. Therefore numbers of impulses in the first 1 sec after stimulation was measured and was taken as a measure for representing the response magnitude.

RESPONSE OF WARM- OR COLD-TASTE UNITS TO SOLUTIONS OF VARYING TEMPERATURES

Responses obtained from a single unit to 1/2 M NaCl, 1/256 M quinine or 1/256 M HCl of varying temperatures were rather variable. Some units showed maximal response at 30°C, as shown in Fig. 7, while in other units response was not maximal at 30°C, as shown in Fig. 10. In this figure responses of two units to 1/256 M quinine are shown. The unit of large spike height shows decreasing frequency with a rise in temperature, while the unit of small spike shows the maximal frequency at 30°C. This variable response of units to solutions of varying temperature may be attributed to (i) variable sensitivity of units to taste stimuli and (ii) the fact that any units respond to both taste and thermal stimuli.
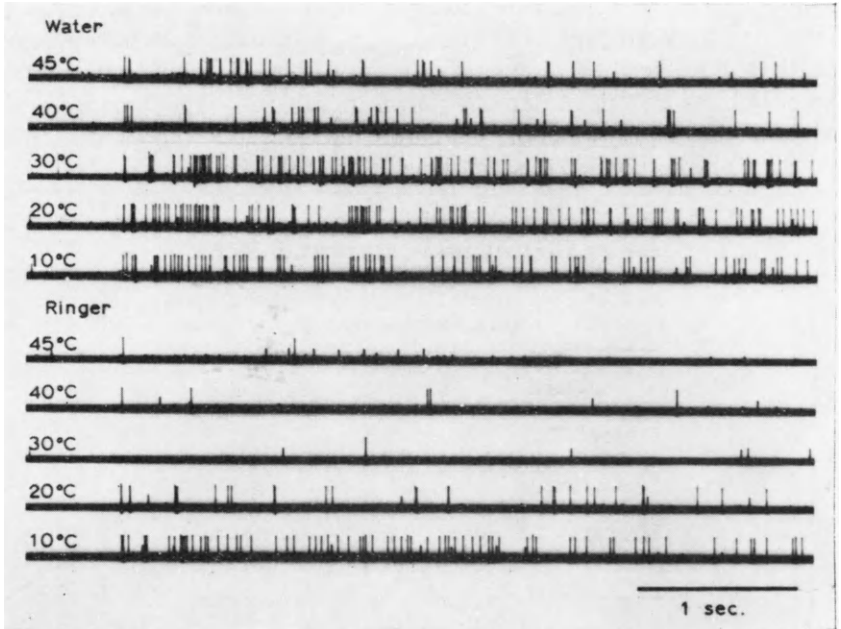
FIG. 8. Impulse discharge of a water-sensitive unit (large spike) to water of 10–45°C, and to Ringer's solution of 10–45°C. Note that this unit is sensitive to cooling. From Nagaki, Sato and Yamashita, unpublished.
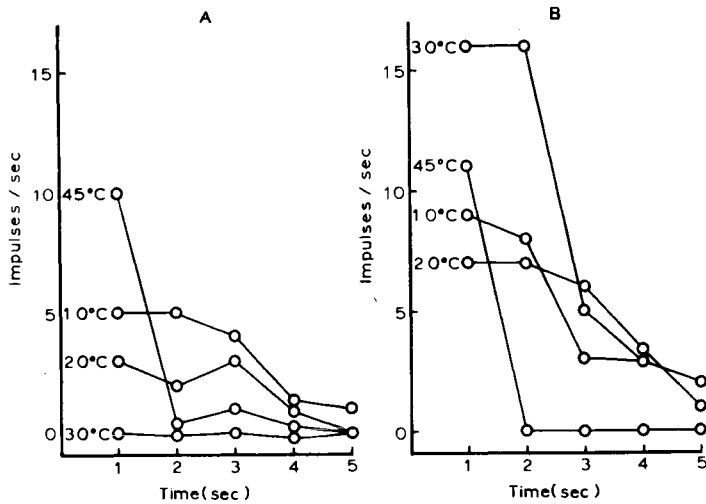
FIG. 9. Adaptation of the response of a quinine-sensitive unit to Ringer's solution (A) and to 1/256 M quinine (B). Abscissa indicates the time elapsed after application of solutions. Numbers attached at each curve show temperature of solutions. From Nagaki, Sato and Yamashita, unpublished.

Therefore, not only the temperature but also the concentration of taste solutions were changed as a next step. One example of an experiment on the NaCl-sensitive unit is shown in Fig. 11. Numbers of impulses discharged is increased by raising or lowering the temperature of Ringer's solution. When NaCl concentration is low, the curve relating the impulse frequency to the temperature is similar to that obtained with Ringer's solution, showing the minimal response at 30°C. However, by increasing
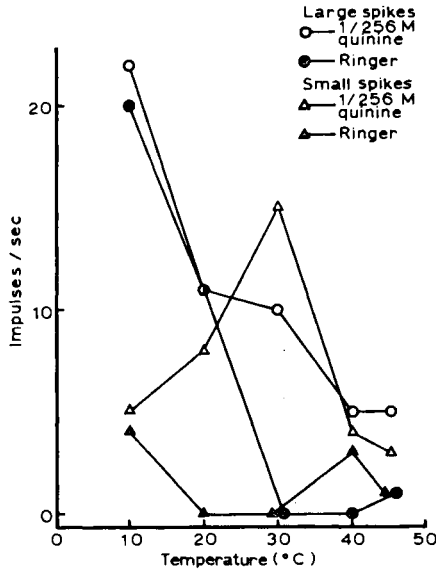

FIG. 10. Impulse frequency–temperature relationships of large and small units, obtained from the same experiment. Ordinate ; number of impulses in the first second, abscissa ; temperature of solutions. From Nagaki, Sato and Yamashita, unpublished.

the concentration, the response to the solution of 30°C becomes increased prominently, and with 1 M NaCl the response shows the maximum at 30°C. This is more clearly shown in the righthand figure of Fig. 11. It is seen that increase in the impulse frequency with an increase in the concentration is greatest at 30°C, and it becomes reduced with a rise or fall of the temperature. Response of a quinine-sensitive unit to quinine solutions of varying temperatures and of varying concentrations is shown in Fig. 12. It shows similar findings to those presented in Fig. 11. All the experiments on single taste units, in which both concentration and temperature of solutions were changed, yielded similar results to those in Figs. 11 and 12. Results obtained with single taste units are essentially the same as those obtained with the whole chorda tympani, and indicate that sensitivity to taste stimuli is greatest when the temperature of solutions is 30°C or at the temperature of the tongue.

FIG. 11.   Relationship between number of impulses and temperature of NaCl solutions (A) and that between number of impulses and NaCl concentration (B), obtained with a NaCl-sensitive unit.   Numbers attached at each curve in B indicate temperature of solutions.   From Nagaki, Sato and Yamashita, unpublished.
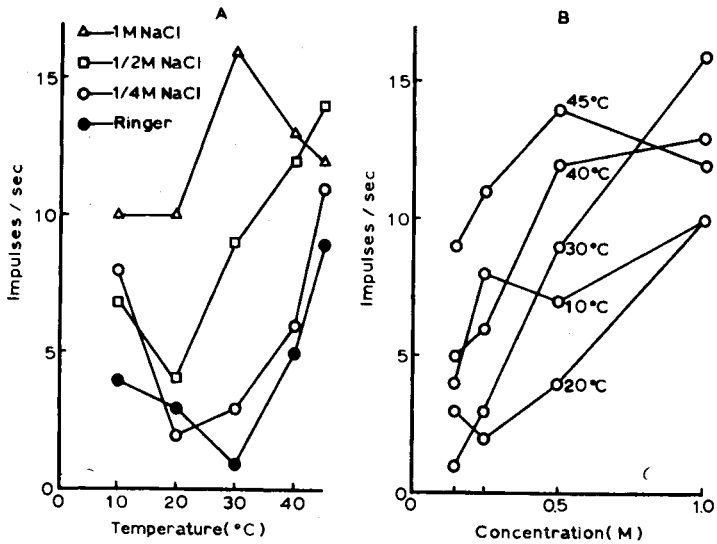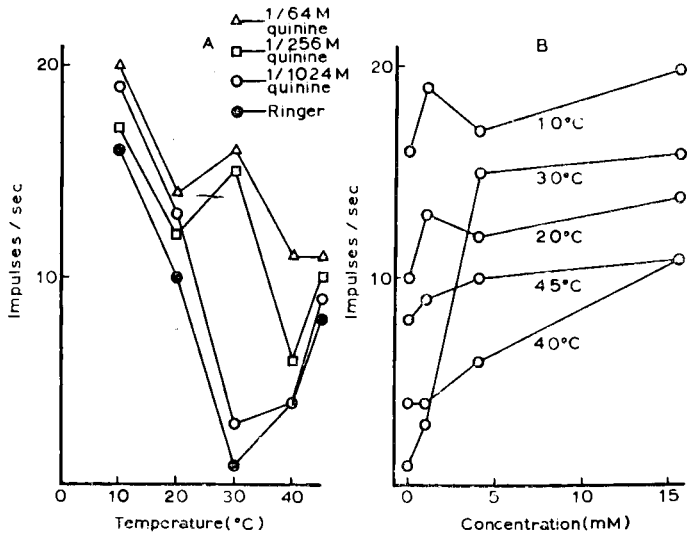

FIG. 12.   Relationship between number of impulses and temperature of quinine solutions (A) and that between number of impulses and quinine concentration (B), obtained with a quinine-sensitive unit.   Numbers attached at each curve indicate temperature of solutions.   From Nagaki, Sato and Yamashita, unpublished.

COMPARISON OF THE RESULTS ON CATS WITH THOSE ON HUMAN SUBJECTS AND ON FROGS

Our results shown above are not consistent with the work by Hahan (1936), but are in rather good agreement with the classical work by Weber (1847). Recently Shimizu, Yanase and Higasihira (1959) investigated the relationship between taste sensitivity of 27 Japanese girls aged 20–22 years old and temperature of taste solutions. Their results are summarized in Fig. 13. Most of the subjects showed the maximal sensitivity to NaCl and sucrose at the temperature of 30–40°C, and nearly half of the subjects showed the maximal sensitivity to quinine and tartaric acid at about 35°C although the remaining half showed declining sensitivity to quinine and tartaric acid with increasing temperature of solutions. Therefore our results on cats are in good agreement with those on human taste sensations by Weber and by Yanase *et al.*, although the optimal temperature in cats is about 30°C while that in human taste sensation is higher than 30°C. The difference is possibly attributed to higher temperature in the human tongue than that in the tongue of anaesthetized cats.
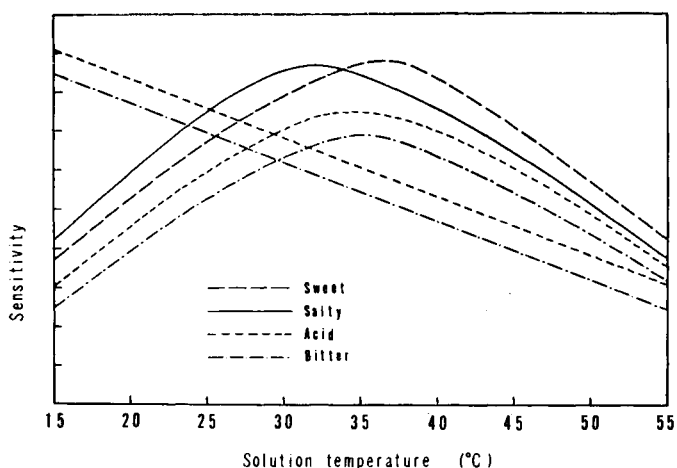
FIG. 13. Relationship between taste sensitivity of human subjects and temperature of taste solutions. The curves are drawn schematically from results on 27 Japanese girls. From Shimizu, Yanase and Higasihira, 1959.

Kimura (unpublished), recording impulse discharges in single nerve fibres in the glossopharyngeal nerve of frogs, and Yamashita (unpublished), recording the integrated response of the whole glossopharyngeal nerve, investigated the relationship between magnitude of the neural response and temperature of taste solutions. Results by Yamashita show that there is no optimal temperature for taste sensitivity in frogs. In Fig. 14 is shown an example of the response of the glossopharyngeal nerve to stimulation

of the tongue with NaCl of varying concentrations and temperatures. The response magnitude is increased gradually with a rise in temperature (left in Fig. 14). This is probably attributed to increase in the response of units sensitive to warming, because in frogs there are no cold units but there are warm units (Kimura, unpublished). The response magnitude is increased with an increase in the concentration, but the increment of the response magnitude is nearly constant from 5°C to 30°C, although it is reduced slightly above 35°C (right in Fig. 14). The response to other kinds of taste stimuli is more or less the same as that to NaCl.
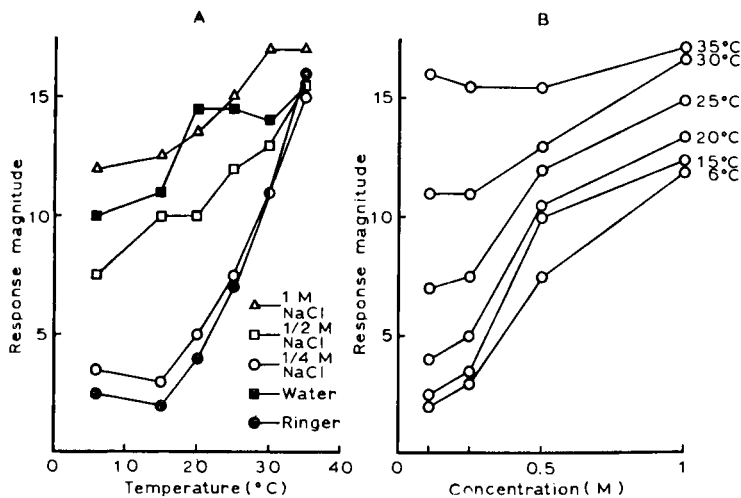
Fig. 14. Relationship between the magnitude of the integrated response of the glossopharyngeal nerve in a frog and temperature of NaCl solutions (A) and that between the response magnitude and NaCl concentration at varying temperatures (B). From Yamashita, unpublished.

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CHEMICAL STRUCTURE AND STIMULATION BY CARBOHYDRATES*

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ABSTRACT

Based on considerations of the configurational and conformational structure of polyols and experimental evidence, it is proposed that the polyols which stimulate taste receptors of the blowfly combine with two or more distinct receptor sites, each with unique structural requirements, and that these sites are associated with the same receptor cell. The stimulating effectiveness of a series of derivatives of D-glucose are assessed to determine the structural requirements for stimulation by that molecule. It is tentatively concluded that only the hydroxyl groups on C_3 and C_4 of the molecule combine with the receptor site and that additional specificity is conferred on the reaction by steric hindrance due to substituents not otherwise involved. Some evidence is discussed in relation to the mechanism of stimulation by polyols. It would appear that polyols combine with receptor sites through weak, physical forces although the reaction has a high degree of specificity. How the reaction produces depolarization of the receptor is posed as a unique and challenging problem of neurophysiology.

INGESTIVE behavior of animals is frequently elicited upon stimulation of their taste receptors by various carbohydrates. The chemical species which evoke a "sweet" taste for mammals, and especially humans, have been the subject of much research (cf. Pfaffman, 1959). There are a number of puzzling facts about the sweet taste, probably the most puzzling of which is the wide array of chemical species that elicit it, while at the same time there is a rather high degree of specificity sometimes toward the structure of chemicals of the same general type. Many carbohydrates (e.g. glycols, glucose, sucrose, and glycogen), L-amino acids (not D), certain synthetic aromatic compounds (e.g. saccharin), lead acetate, and beryllium salts, all are effective. Furthermore, the same inhibitor for the sweet taste (gymnemic acid) blocks stimulation by radically different chemicals (sucrose and saccharin : Warren and Pfaffman, 1959). There are also complexities relating to the frequent close association of different taste qualities (sweet and bitter), both in terms of chemical structure and after-tastes. Gymnemic acid inhibits both sweet and bitter, while the salt and acid qualities are unaffected. And synthetic sweeteners, such as saccharin, often has a bitter after-taste. The last thorough attempt to relate sweetness to chemical

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structure in man was that of Oertly and Myers (1919), and that theory is inadequate if only because it does not encompass all of these effective chemicals.

Responsiveness to carbohydrates has been studied extensively also among insects, in several cases with a view to relating stimulating effectiveness to chemical structure. Most of this work, like that in mammals, has employed behavioral criteria of stimulation. Two especially thorough studies were those of von Frisch (1935) on the honeybee and Dethier (1955) on the blowfly, *Phormia*. There are a number of striking differences between the " sweet " taste in these insects and in mammals, most important of which is the extreme specificity of the insect receptors. Only certain carbohydrates are effective (water was erroneously thought to be, but see Mellon and Evans, 1961, and Evans and Mellon, 1962a). Although numerous chemicals have been assayed behaviorally and also electrophysiologically, amino acids, inorganic salts, many organic compounds including saccharin and dulcin, and even most carbohydrates are not effective stimuli.

With regard to stimulation by various carbohydrates then, Dethier (1955) agreed with von Frisch (1935) that only a few limiting generalizations were possible. The main conclusions for the blowfly were : (1) The size of the molecule is important. Shorter carbon chains than pentoses were ineffective ; some mono- and di-, and tri-saccharides were effective ; and the few polysaccharides tested were ineffective. No oligosaccharides larger than tri-saccharides were examined. (2) Linear polyols are ineffective ; for example, D-glucitol is tasteless in contrast to glucose. However, Haslinger (1935) found that another blowfly, *Calliphora*, responded to polyhydric alcohols when starved ; and Evans (see below) obtained responses of starved *Phormia* to saturated solutions of D-glucitol. Linear polyols probably are, therefore, weakly effective. (3) The carbonyl group is not required for stimulation. *Myo*-inositol and non-reducing glycosides are stimulating. (4) *Alpha* glucosidic derivatives (carbohydrate or other) are generally much more effective stimuli than are otherwise similar *beta* derivatives.

Both Dethier and von Frisch attempted unsuccessfully to discover configurations that were common to the stimulating carbohydrates and were absent in the tasteless ones. Even when only the 5 and 6 carbon aldoses were taken into account, there was no apparent relationship between stimulating effectiveness and configuration.

The present paper represents a re-evaluation of the earlier data of Hassett *et al.* (1950) and Dethier (1955) for *Phormia* and presents some new data and a theory that attempts to explain why stimulating effectiveness could not formerly be related to chemical structure. In addition, some evidence is discussed as it pertains to the mechanism of stimulation by carbohydrates, or better, polyols.

MATERIALS AND METHODS

All of the data refer to the usual behavioral assays for sugar sensitivity used by Dethier (1955 and earlier) on the adult blowfly, *Phormia regina* Meigen. Much of the data to be discussed is that of Hassett *et al.* (1950) and Dethier (1955). For the present purposes some new compounds were obtained and assayed. The sensitivity of a population of 30 to 120 flies was assessed for each new compound in relation to the sensitivity of that population to an equilibrium solution of glucose.

Compounds 1 and 2 in Table 1 had previously been tested by Dethier (1955), but were retested. *Alpha* glucose is the usual commercial form, and *beta* glucose was prepared by crystallization from hot acetic acid (m.p. = 149°C). The *gamma* and *delta* lactones of gluconic acid were purchased (Pfansteil). Each of the four compounds mentioned were examined for purity and rate of mutorotation by polarimetry. These compounds were then assayed on the blowfly under similar conditions soon enough after solution in water so that 98+ per cent of the solute was in the desired form as calculated from the polarimetric data. Gold thioglucose was given by the Scherring Research Division and was pure according to chromatographic examination in three solvent systems (Evans and Dethier, 1957). Polygalitol and arlitan were prepared from a sample given by the Research Division of the Atlas Powder Company. An aqueous solution containing between 80 and 85 per cent of a mixture of glucitol, polygalitol, and arlitan was provided. Chromatography of the sample separated the three compounds. Glucitol was identified by its migration with authentic glucitol (sorbitol–Pfansteil). The two anhydrides were identified by their differential reactivity with periodate (Evans and Dethier, 1957). Chromatography on thick electrophoresis paper and subsequent elution provided large enough samples of the pure materials for assay. 2-deoxy-, 2-0-methy-, and 3-0-methyl-glucose were a gift from Dr. N. K. Richtmeyer. Mono- and di-isopropylidene glucose (1,2 and 1,2,5,6 mono- and di-acetone glucose respectively) were prepared according to Mehltretter *et al.* (1951). The melting points were 159–162 and 108–110°C respectively. Dethier (1955) had reported that diacetone glucose was stimulating. From theoretical considerations and later tests of the authentic material (see below), this seemed highly improbable. Accordingly, the melting point of the " diacetone glucose " used by Dethier was determined ; it was 150–153°C in contrast to 110–111°C of the pure material (Bates *et al.*, 1942).

All of the polyols, sugars, and sugar derivatives referred to below are of the D-series unless stated otherwise or the designation is not applicable.

RESULTS AND DISCUSSION

Multiple Combining Sites for Polyols

The initial stimulus for the present study derived from the unsatisfying

conclusions of Dethier (1955) and von Frisch (1935) that chemical struc-
ture could not be related to stimulating effectiveness. These authors had
considered only configurational structure. However, even when the con-
formation of the molecules was taken into account, it was apparent that a
single combining site could not account for the data. There had to be
multiple combining sites with different structural requirements to account
for the extreme structural specificity on the one hand and the odd array of
effective molecules on the other.

The first suggestive observation was that mixtures of the very weakly
stimulating sugar, mannose, with fructose markedly reduced stimulation
by the fructose. Similar mixtures of glucose and mannose were as effective
as if the glucose were present alone (Dethier, 1955). Subsequently,
Dethier *et al.* (1956) reported that mixtures of glucose and fructose were
more effective than their concentrations accounted for, i.e. that their
action in mixtures was synergistic rather than merely additive. And the
inhibition of fructose stimulation by mannose was competitive. The sim-
plest interpretation of these data is that glucose and fructose stimulate by
combining with different sites and that mannose combines ineffectively with
the fructose site, competing with fructose for that site.

Confirmation of this viewpoint came from an unusual source. It was
found that the sensitivity of the adult receptors either to fructose or
to glucose could be altered drastically by rearing the developing larva in
the presence of one or the other sugar (Evans, 1961). The sensitivity to the
two sugars was clearly dissociable experimentally.

This postulation of multiple combining sites explains simply why for-
merly it had not been possible to find a structure that was common to the
stimulating sugars and absent in the tasteless ones. Now two possible
experimental approaches were to determine from studies of mixtures which
sugars or derivatives were inhibitory, synergistic, or merely additive in their
effects or to introduce single small structural changes into one molecule
and assay the effect. The latter, by reason of directness and simplicity was
the method of choice and could show what chemical structure was required
of a molecule for stimulation.

A number of considerations determined which molecule was the most
appropriate for study. In recent years, it has become increasingly evident
that the conformation of sugars, in addition to configuration, plays a fun-
damental role in their chemical and biological reactions. For example,
cis and *trans* in a configurational sense is an almost useless concept in the
reactions of almost all sugars. As has been shown both theoretically and
experimentally (cf. Reeves, 1950, 1958), almost without exception sugars
and their cyclical derivatives (6-membered) assume either one or both of
the two chair forms rather than the boat forms ; and it is only in the boat
forms that true *cis* orientation is possible. The geometry assumed by a

sugar depends upon nonbonded interactions among its substituents : especially the ring oxygen atom, the hydroxyl groups, and the carbinol group of aldohexopyranoses. Of the aldohexoses, only D- and L-glucose are capable of having all such substituents in the equatorial position (Cl and 1C conformations respectively—Fig. 1) where they are most stable.
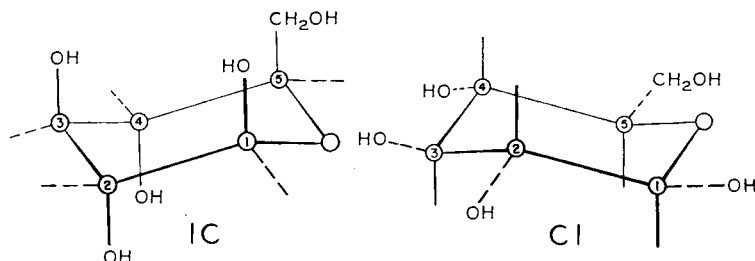

FIG. 1. β-D-glucopyranose in the 1C and Cl conformations.

Hence, both are present in solution (or in crystals) almost entirely in a chair conformation as the aldopyranose and are more stabilized in this form than are other aldohexoses. As a consequence also, derivatives of most kinds do not change the conformation. Based on these considerations, the structure of D-glucose that was responsible for stimulation was assessed by comparing the effectiveness of a series of its derivatives (Table 1). In that table, cpds 1–23 are relevant to the point at hand. Compounds 1–8, 10, and 13–20 have 6-membered rings in the Cl conformation. Compounds 9 and 11 have 5-membered rings which are planar. Compound 12 can be considered largely comparable to glucopyranose by appropriate designation of the first carbon atom. Compounds 21–23 are disaccharides of glucose to which more complicated conformational considerations must be applied.

In several biological systems, conformational analysis has proven essential for an understanding of reaction mechanisms.

LeFavre and Marshall (1958) have given evidence that transport of sugars by the facilitated diffusion system of the red blood cell has simply the requirement that the sugar be in the Cl conformation. The more stable a sugar is in that form, the greater its affinity for the " carrier " system. Hence, D-glucose has about the greatest affinity, and L-glucose the least. Since sugars which are most stable in the 1C conformation are apparently never utilized by organisms, the authors further suggest that enzymes that act on carbohydrates have foremost a requirement for the Cl conformation like the red cell system, but that they then are differentiated by their additional configurational requirements. This generalization does not apply to stimulation of the sugar receptors of the fly since sugars in the 1C conformation are effective stimuli (L-fucose and L-xylose : Hassett et al., 1950).

Conformational analysis has been extensively employed in the study of the requirements of bacterial enzymes that act on cyclitols; and the cyclitols which occur naturally, it has been pointed out, all possess a stable chair form (cf. Angyal and Anderson, 1959).

So far evidence has been given to the effect that all carbohydrates that stimulate the blowfly chemoreceptor do *not* do so by binding to a single combining site. Nothing, however, has been said as to whether the sites are associated with a single sugar receptor cell or more than one. For most of what follows the point is probably irrelevant, but there is evidence that the combining sites belong to the same neuron. Dethier and Rhoades (1954) and Dethier *et al.* (1956) found that flies could not distinguish one sugar from another. Hodgson (1957) tested a number of sugars electrophysiologically and reported that they all activated one receptor cell. Evans (unpublished) assayed solutions of mixtures of glucose and fructose as well as these sugars individually, using the electrophysiological method of Morita (1959), and obtained in each case only the impulses of the water receptor (Evans and Mellon, 1962a) and a single sugar receptor. Thus, the evidence points to a single sensory neuron with two or more types of combining sites for sugars.

Structural Requirements of the Receptor Site for Glucose

In considering the combination of glucose with its receptor site, attention immediately focuses on the polar groups (the hydroxyls, the C_6 carbinol, and the ring oxygen). While almost all of the molecules of glucose in solution are in the pyranose form and Cl conformation, this by itself does not prove that that is the form which reacts with the receptor; but other considerations do. Dethier (1955) could detect no difference between α and β glucose. These experiments were repeated under conditions where less than 2 per cent of the original stereoisomer had mutarotated; again no difference was found in stimulating effectiveness (cpds. 1, 2 and 3; Table 1). On the other hand, the respective glucosides with small aglycons (cpds. 4,5,6,7) were for all intents and purposes qualitatively different. The α-methyl and α-phosphate derivatives were slightly more stimulating than glucose, while the β-methyl and β-gold-thioglucose were ineffective at any concentration. These effective compounds are all confined in the α-pyranose form, so clearly the 6-membered ring was as effective as glucose. Consequently, small amounts of the free aldehyde or furanose form were not responsible for the stimulation by glucose solutions.

The foregoing results could be interpreted either as that the α-oxygen atom was required for stimulation or that β substituents of a certain size hindered stimulation. Compound 8, lacking the C_1 hydroxyl group, was as effective as glucose, indicating β steric hindrance was the case. Furthermore, it showed that a 6-membered ring containing the very stable ether

TABLE 1. STIMULATING EFFECTIVENESS OF SOME CARBOHYDRATES OF THE D-SERIES

Number	Compound Name	Structure	Effectiveness relative to glucose ($=1.0$)
1	α-glucopyranose	C_1—OH α	1.0
2	β-glucopyranose	C_1—OH β	1.0
3	Equilibrium glucopyranose	63% β	1.0†
4	α-glucose-1-PO_4	α—C_1—O—PO_3K_2	1.3†
5	α-methyl glucopyranoside	α—C_1—O—CH_3	1.9†
6	β-methyl glucopyranoside	β—C_1—O—CH_3	—†
7	β-gold thioglucose	β—C_1—S—Au	—
8	1,5-anhydroglucitol (polygalitol)	C_1 deoxy $C_{1,5}$ ether ring	1.0
9	1,4-anhydroglucitol (arlitan)	C_1 deoxy $C_{1,4}$ ether ring	—
10	1,5-gluconolactone	C_1OOH $C_{1,5}$ ester ring	1.0
11	1,4-gluconolactone	C_1OOH $C_{1,4}$ ester ring	—
12	*myo*-inositol	C_1—OH β C_6HOH replaced by HOH (equitorial) ring O replaced by CHOH (axial)	0.7†
13	2-deoxy glucopyranose	C_2 deoxy	0.8
14	2-0-methyl glucopyranose	HC_2—O—CH_3	0.5
15	glucosamine	HC_2NH_2	0.6†
16	mannopyranose	C_2—OH axial	0.02†
17	3-0-methyl glucopyranose	HC_3—O—CH_2	—
18	gulopyranose*	C_3—OH axial	—†
19	xylose	HC_6OH replaced by H	0.30†
20	glucose-6-PO_4 (Ba)	HC_6—O—PO_3Ba	1.6†
21	maltose	4—O— α-glucopyranosyl-glucopyranose	33‡
22	trehalose	α-glucopyranosyl— α-glucopyranose	1.0†
23	cellobiose	4—O—β-glucopyranosyl-glucopyranose	0.026†
24	fructose		21.6†
25	sucrose	α-glucopyranosyl—β-fructofuranoside or β-fructofuranosyl— α-glucopyranoside	13.2†
26	glucose-fructose mixture (equimolar)		17†
27	turanose	3—O— α-glucopyranosyl-fructofuranoside	12†
28	melezitose	O— α-glucopyranosyl—(1——→3)— O—β fructofuranosyl—(2——→1)— α-glucopyranoside	2.1†

*impurities present.   —no response at any stimulus intensity.
†reported or cited in Dethier (1955).

linkage was effective, while the same molecule with a 5-membered (planar) ring (cpd. 9) was inert. Compounds 10 and 11 point to essentially the same conclusion.

That the ring oxygen atom itself is not required for stimulation is suggested by cpd. 12, *myo* inositol. Since this molecule is so different from glucose, it was necessary to obtain evidence that it stimulated by combination at the glucose site. Glucose alone and several mixtures of glucose and inositol of varying properties were tested on 120 animals. Responses to the mixtures averaged 88 per cent of the responses to glucose solutions of equivalent concentration, close to the value that would be expected were the compounds strictly additive in their interaction with the receptor. In addition, an axial hydroyl group on the ring of inositol where the pyranose oxygen atom usually is located did not interfere with stimulation.

Removal of the C_2 oxygen, methylation of it, or replacement with an amino group had little effect on stimulation (cpds. 13–15) ; but switching the hydroxyl group from the equatorial position to axial rendered it 50 times less effective (cpd. 16). As mentioned before, glucose and mannose are strictly additive in mixtures.

Truly appropriate compounds have not yet been obtained to assess the requirement for the C_3 hydroxyl group (e.g. 3-deoxy glucose). Methylation (cpd. 17) or transfer to the axial orientation (cpd. 18) render the molecule ineffective, but the former adds on a larger substituent that might sterically hinder the reaction and pure gulose has not been assayed.

Appropriate tests have not been made of the C_4 and C_5 substituents, but the latter normally is part of the pyranase ring and has otherwise only a hydrogen substituent. Galactose (C_3-OH axial) stimulates (effectiveness relative to glucose $= 0.26$), but there is no evidence yet that it reacts with the glucose site.

The C_6 carbinol group appears unimportant since it is lacking in inositol and xylose and since relatively large esteratic substituents (cpd. 20) do not interfere.

In summary, the evidence is consistent with the view that the hydroxyl groups on C_3 and C_4 alone are responsible for stimulation. They are *trans*, but in the Cl conformation are respectively inclined about 19° above and 19° below the plane of the ring (see Fig. 1). A two point attachment seems incompatible at first with the degree of specificity the receptor exhibits, but the marked interference noted above by substituents otherwise not involved could contribute to that specificity.

In accordance with the above considerations is the observation that the synthetic mono- and di-isopropylidene glucose derivatives are non-stimulating—the compound Dethier (1955) tested probably was not diacetone glucose as indicated above in "Materials and Methods". Consequently, his conclusion that furanose forms of glucose can stimulate appears incorrect.

If the structural requirements are as proposed, it might be expected that linear polyols would stimulate ; but Dethier (1955) reported that mannitol and glucitol did not. Some small fraction of *vicinal* hydroxyl groups, however, ought to be in the proper orientation in solution at any given time. When flies were starved longer than Dethier's, until they were maximally sensitive, all would respond to saturated solutions of the two hexitols. And further, ingestion of the solutions, which depends on continual stimulation of the sugar receptor (Dethier *et al.*, 1956), was supported for 1–2 min. While taste thresholds measure the inital response of the sugar receptor (i.e. for *ca.* 100 msec), feeding duration is a measure of the continuing response. Since the hexitols are free to rotate about single C—C bonds and all conformations are potentially rapidly interconvertible, the receptor may bind one form, causing conversion of others in the solution to more of that form.

Possible Spatial Relationships of Combining Sites

The remaining selected compounds listed in Table 1 may signify something about the size and/or spatial distribution of combining sites. Since maltose is among the most effective sugars ($33 \times$ more effective than glucose), it may be that the combining sites are larger than one hexose unit, or that sites are spatially juxtaposed. Recent evidence suggests that the non-reducing hexose unit of maltose does not have the Cl conformation (Reeves, 1959, pp. 19–20) and the C_4–OH of the reducing moiety is involved in the glucosidic link ; consequently, the great effectiveness of maltose most likely is not owing to the juxtaposition of two similar glucose binding sites. Another α disaccharide of glucose, trehalose, is only about as effective as glucose alone ; while another disaccharide of glucose with the β configuration (cellobiose) is only weakly effective. As was pointed out before, glucose and fructose synergize in mixtures (Dethier *et al.*, 1956 and Table 1). Sucrose is somewhat less effective than such mixtures and much less so than fructose alone. A similar disaccharide of glucose and fructose (turanose) but linked at the C_3 position of fructose is about as effective as sucrose. The trisaccharide, melezitose, which corresponds to either sucrose or turanose with a glucose residue linked to one or the other end is much less effective than either of the disaccharides. Very likely these and other similar results reflect the size and arrangement of combining sites on the receptor and the conformation of the molecules.

Mechanism of Stimulation

Dethier (1955) has given evidence that stimulation by sugars involves a physical rather than a chemical reaction, but, of course, a highly specific one. His argument was based largely on the lack of effect of various metabolic inhibitors on sugar stimulation (e.g. azide, fluoride, cyanide, iodo-

acetate).   Strongly stimulating sugars (i.e. L-fucose = 6-deoxy-L-galactose) could be fed to an animal confined in a glass vial and absorbed into the hemolymph and then quantitatively recovered 24–48 hr later from the animal and vial without chemical alteration (Evans, cited in Dethier, 1956). L-fucose also has been shown to be unable to sustain life of the adult fly in contrast to utilizable sugars (Hassett *et al.*, 1950).   Hence, in at least this case, the animals lacked enzymatic equipment for chemical conversion of the sugar (see also Evans and Dethier, 1957).   On the other hand, this evidence does not necessarily show that the differentiated sugar receptor also lacks the ability to produce a chemical change in the fucose molecule.

The most convincing evidence for a physical reaction derives from the behavioral tests of Dethier and Arab (1958) which showed that a change of temperature of about 0–35°C in the stimulating solution did not affect stimulation when the solution contacted only the tip of the chemosensory hair.   A number of earlier reports had shown that the temperature of the receptor cell body or the whole animal, as might be expected, did influence the behavioral response to sugars.   Consequently, the primary process in stimulation by sugars is very likely not influenced by temperature over a very wide range.

Recent measurements of the latency of the response of the sugar receptor showed that the first impulse can occur at least as soon as 4 msec after application of the stimulus (Browne and Hodgson, 1962).   It may even occur much sooner since these authors measured the latency to sugars contained in a saline solution.   Since it is a common observation that the initial frequency is greater than any subsequent frequency, this evidence indicated that the stimulus is fully effective in a very short time.   In this period it is generally accepted that sugar depolarizes the tip of the receptor cell and that the depolarization spreads electrotonically to the cell body near which the impulse is generated.   Morita and Yamashita (1959) have claimed in addition that the impulse is propagated in a non-decremental manner distally as well as proximally from the region of spike generation, but their evidence is not conclusive.   In any case, the stimulus is fully effective in a brief period in which a number of events must occur ; it would seem that the receptor sites are immediately accessible to the stimulating molecules, i.e. that they are associated with the cell surface rather than inside any barrier.

Prospects

The theories and evidence above are based upon behavioral evidence, and now should be checked by electrophysiological studies of the sugar receptor.   Quantitative analyses have been made of the water (Evans and Mellon, 1962a) and the salt (Evans and Mellon, 1962b) receptor cells, and using the recording method of Morita (1959) can now be extended to

the sugar receptor cell. Hodgson (1957) reported preliminary experiments to this end, but his methods were inadequate. He assayed a number of sugars, but they were always dissolved in a NaCl solution. It has been shown that some ions influence the response of the sugar receptor (Evans, 1958 ; Tateda and Morita, 1959), as might very well have been expected. Furthermore, since he believed there were only two spike types in recordings from the preparation, when in reality there are potentially four (Evans and Mellon, 1962a) or more, his counts of the " sugar spikes " cannot be taken at face value, and should be repeated using methods that permit assignment of a given spike in a record to a particular sensory cell.

When appropriate quantitative electrophysiological studies are made, the mechanism of stimulation by polyols may be elucidated. If as is purported, the reaction of sugar with receptor site does not involve chemical changes in the molecule, the mechanism promises to be a unique neural process. While potential changes of membrane produced by fat soluble molecules and ions are familiar, depolarization of a membrane by a highly specific physical combination with un-ionized, water-soluble sugar molecules promises to involve interesting processes. Since individual mammalian taste bud cells respond to an array of chemical species even broader than those which elicit a sweet taste (Kimura and Beidler, 1961), the sugar receptor cell of insects is the more advantageous preparation for this purpose.

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ELECTROPHYSIOLOGICAL RESPONSES TO SUGARS AND THEIR DEPRESSION BY SALT

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SEVERAL attempts have been made to relate sweet taste to certain molecular configurations. Well known for instance is the classification by Cohn (1914) of so-called " sapophoric " groups including, among others, multiple hydroxyl groups in certain molecules and the α-amino groups in the amino acids. Another systematization which has been frequently referred to is that of Oertly and Myers (1919), in which these authors, under apparent influence of de Witt's theory for dyestuffs, postulated that a " glucophore " and an " auxogluc " are required in a molecule in order to produce sweet taste. However, because of the many and important exceptions from the postulated rules, none of the theories relating sweet taste to molecular configurations is comprehensive.

Agents which taste sweet to humans are found not only among the organic compounds, but include certain inorganic salts as well. However, even if we limit our discussion to only the organic substances eliciting sweet taste, any classification based on structural characteristics of the stimulating molecules will necessarily encounter serious difficulties. Firstly, because the sweet-tasting organic compounds belong to a wide variety of chemical groups characterized by entirely dissimilar molecular skeletons. Secondly, because increasing molecular weight within a homologous series may be accompanied by a change in taste from sweet to bitter ; and finally, because a single substitution into a complex molecule with one very simple group may have a striking effect on altering the taste of the original compound (Hamor, 1961).

It appears, therefore, that our present knowledge is insufficient to support a comprehensive classification of sweet tasting compounds based on their molecular configuration, and one may question whether such a systematization will ever become feasible. The complexity of the situation outlined above, it seemed to us, called for an investigation on a much simplified

problem and a relatively modest aim, namely, study to the stimulating ability of a series of intimately related substances and, if possible, to correlate this parameter with at least one physico-chemical property of the compounds used. For this purpose we selected three stereo-isomeric aldohexoses D-galactose, D-glucose and D-mannose, two ketohexoses D-fructose and L-sorbose ; and in addition three disaccharides, namely, sucrose, maltose and lactose were tested.

During the investigation we became interested in the interaction of gustatory stimuli on the peripheral level, and carried out a second series of experiments in order to study this phenomenon with respect to salt and sugar. This paper accordingly falls in two parts dealing with sweet stimulation, and the peripheral interaction between sweet and salty stimuli, respectively.

METHODS

Mongrel dogs were used in the experiments. Because all of the four classical taste qualities, as well as the water taste, are mediated through the chorda tympani in the dog (Appelberg, 1958), only fibres running in this structure were studied.

Detailed information about the experimental procedure has been furnished in a series of previous publications and will not be repeated here (Zotterman, 1936 ; Liljestrand and Zotterman, 1956 ; Kitchell et al., 1958 ; Gordon et al., 1959 ; Konishi and Zotterman, 1961).

The receptors of the tongue were stimulated with 0.5 M solutions of the monosaccharides D-galactose, D-glucose, D-mannose, D-fructose and L-sorbose, and the three disaccharides sucrose, maltose and lactose, which are α-D-glucopyranosyl-β-D-fructofuranoside, α-D-glucopyranosyl-D-glucopyranose and β-D-galactopyranosyl-D-glucopyranose, respectively (Fig. 1).

Fresh samples of the solutions were made up 2–3 hr before use, so that fermentation was negligible, whereas mutarotation would reach equilibrium. Throughout the experimental period the solutions were kept in a water bath which was maintained at 35–36°C. The temperature receptors, therefore, remained silent when the sapid solutions were applied to the tongue. Similarly, precautions were taken as to the arrangement of the dispensing apparatus, so that no nervous discharge from the mechano-receptors was elicited when the test-solutions were poured on to the surface of the tongue.

For the purpose of the present investigation the specificity of the preparations studied was tested with water, 0.5 M NaCl and 0.01 M quinine in addition to the sugar solutions.

In the second series of experiments the following test-solutions were used : 0.5 M sucrose, 0.5 M sodium chloride and a mixture containing 0.5 M sucrose *and* 0.5 M sodium chloride. The concentration of the sodium chloride solution was in one experiment reduced to 0.2 M, and the mixture was adjusted accordingly.
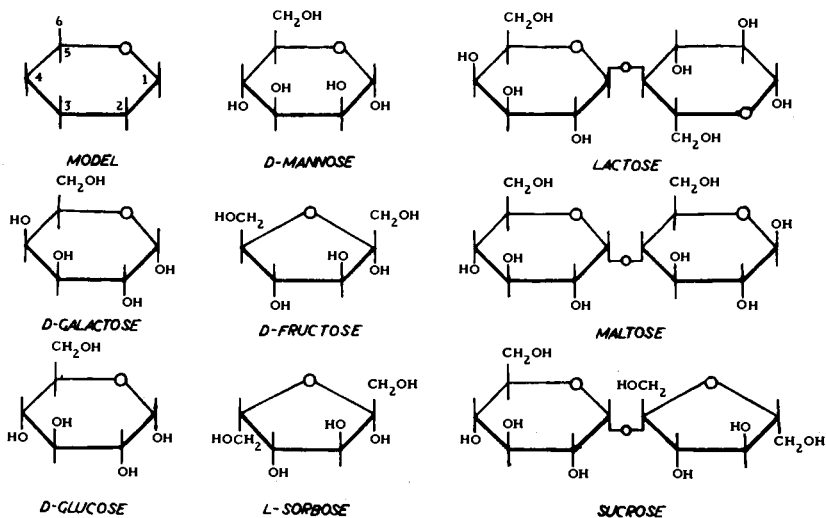

FIG. 1. Structural formulas of sugars investigated.

Altogether 43 functional single fibres from 25 dogs were studied, and the integrated responses from the unsplit chorda tympani were recorded in all of the experiments.

RESULTS AND DISCUSSIONS

A.   Responses to Sugars

1.   *Responses of the whole chorda tympani.*   The integrated responses obtained in one experiment are presented in Fig. 2.   It appears that D-fructose produced the largest response of all of the sugars as measured by the deflection of the integrated beam from the base level.   The other ketohexose, L-sorbose, was roughly half as effective as a stimulus.   D-mannose was the most powerful stimulus of the aldohexoses.   D-galactose and D-glucose produced very nearly the same response in the experiment shown in Fig. 2, but on the whole the latter proved to be the stronger of the two.   The 0.5 M solutions of the three disaccharides gave the following order of decreasing stimulation ability :   Sucrose>Maltose>Lactose, sucrose being the second best stimulus of all of the sugars studied.

We have used the recordings obtained with the integrator for a comparison of our results with the psychophysical data collected from investigations

on human subjects by various other authors. Our figures have been arrived at by calculating an average value for each of the sugars, using all of the experimental data obtained by recording from the whole chorda tympani. In accordance with the practice of the previous investigators the mean response of sucrose has been given a value of 100 arbitrary units, and the averages for the other sugars has been adjusted in relation to this
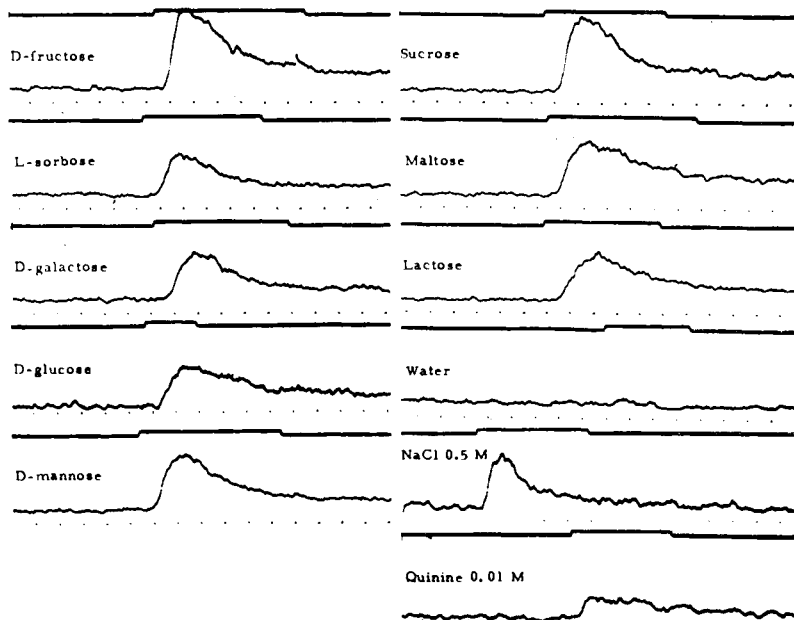

FIG. 2. Electrical responses recorded from whole chorda tympani upon application of 0.5 M sugar solutions to tongue. Responses to 0.5 M NaCl, 0.01 M quinine and water are also shown. From top to bottom of each recording are shown signal from dispensing burette, integrated response and time marks. Time in seconds.

standard. The comparison is shown in Table 1 (p. 183) where our figures are listed together with those of Becker and Herzog (1906), Biester, Wood and Wahlin (1925), Walton (1926) and Fabian and Blum (1943). In the cases where no quantitation has been attempted by the authors the sweetest sugar has been identified with No. I, the next with No. II and so on. These roman numbers are also given in parenthesis after the quantitative ones for the sugars common in all of these investigations.

In order to decide whether D-fructose and sucrose produce such large responses in the unsplit chorda tympani by activating more fibres than the other sugars, or by stimulating the proper receptors with a higher frequency, we have to turn to an analysis of the impulse traffic in preparations containing only one functional fibre.

2. *Individual fibres.* (i) *Monosaccharides.* Upon stimulation with 0.5 M solutions of the monosaccharides we found fibres which were activated by all of the sugars, but also fibres which only responded to the most
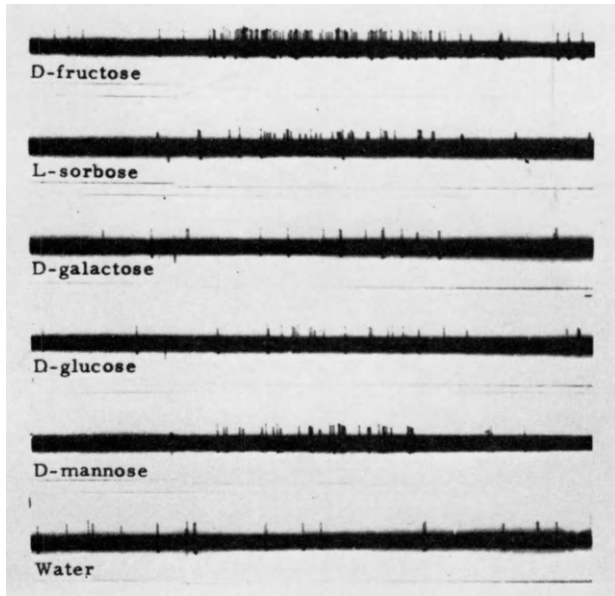

FIG. 3. Records obtained from small strand of chorda tympani containing only one functional fibre. Time in seconds.
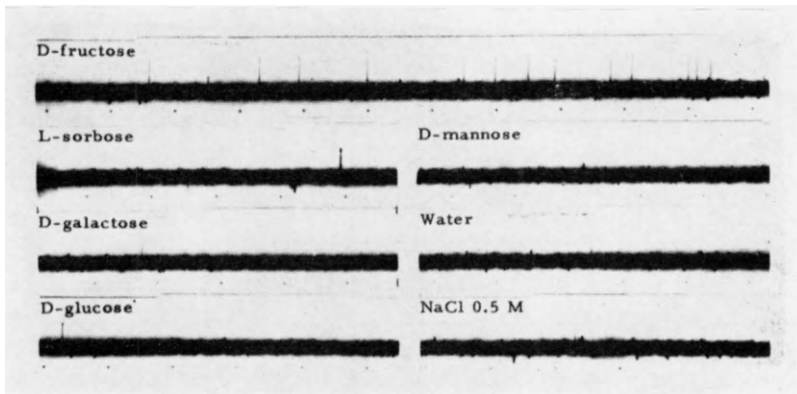

FIG. 4. Functional single fibre from the chorda tympani responding to D-fructose only among the 0.5 M solutions of monosaccharides. Time in seconds.

potent one, D-fructose. An example of the first type is shown in Fig. 3. As usually, D-fructose produced a much more massive response in terms of spikes per unit time than the other sugars. L-sorbose and D-mannose

were equally strong, whereas D-glucose and especially D-galactose elicited relatively small responses. The other type of fibre which was activated only by stimulation with D-fructose is shown in Fig. 4.

Neither one of these two fibres responded to water, 0.5 M sodium chloride or 0.01 M quinine solutions.

(ii) *Disaccharides.* An example of the electrical activity in specific sweet fibres upon stimulation of the tongue receptors with the three disaccharides is shown in Fig. 5. The results obtained confirmed the integrator-recordings, namely, sucrose produced the largest response of these three sugars, lactose the smallest, with that of maltose falling in between. Another
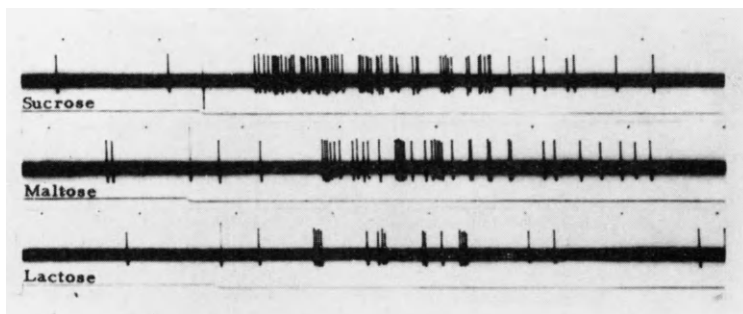

FIG. 5. Electrical responses from single fibre recorded upon application of 0.5 M solutions of the disaccharides to the tongue. Time in seconds.

feature of Fig. 5 which is noteworthy is that the period of time which elapsed from the application of the sapid solutions to the tongue until the response could be recorded was shortest in the case of sucrose.

(iii) *Complete sugar series.* Figure 6 shows the responses recorded after stimulation with the complete sugar series. D-fructose again produced the most conspicuous response in terms of spikes per unit time. Sucrose was second in stimulating ability, next followed by D-glucose, D-galactose, L-sorbose and maltose. Lactose and D-mannose elicited the smallest responses in this experiment.

(iv) *Unspecific fibres.* Certain fibres were activated by a 0.5 M NaCl solution as well as by the sweet stimuli. An example is given in Fig. 7. However, among the sugars only D-fructose and sucrose were able to produce a discharge of electrical activity in this preparation ; that of D-fructose was by far the largest, while sucrose and sodium chloride elicited exactly the same response in terms of spikes per unit time.

We have seen that there is a very good conformity between previous psychophysical findings in human subjects and our electrophysiological data from the dog (Table 1). Whereas the over-all picture is very

TABLE 1. RELATIVE SWEETNESS OR STIMULATION ABILITY OF SUGARS

Sugar	Becker, Herzog	Biester, Wood, Wahlin*	Fabian, Blum	Walton	Andersen, Funakoshi, Zotterman
D-fructose	II	173 (I)	I	103–150 (I)	120 (I)
D-glucose	III	74 (III)	III	50–60 (III)	70 (III)
D-galactose	VI	32 (IV)	—	—	59 (V)
D-mannose	—	—	—	—	77
L-sorbose	—	—	—	—	86
Lactose	V	16 (VI)	V	27–28 (V)	53 (VI)
Maltose	III	32 (VI)	IV	60 (III)	67 (IV)
Sucrose	I	100 (II)	II	100 (II)	100 (II)

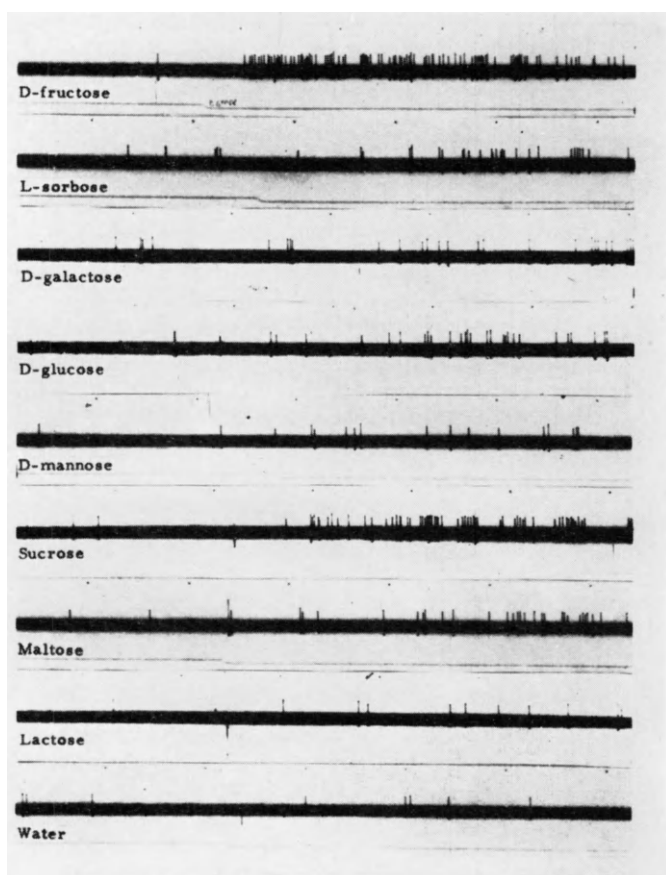* Equal weights of sugars used in test solutions.

FIG. 6. Records from thin strand of chorda tympani containing only one functional fibre. Note the large responses to D-fructose and sucrose as compared to those of D-galactose and lactose. Time in seconds.
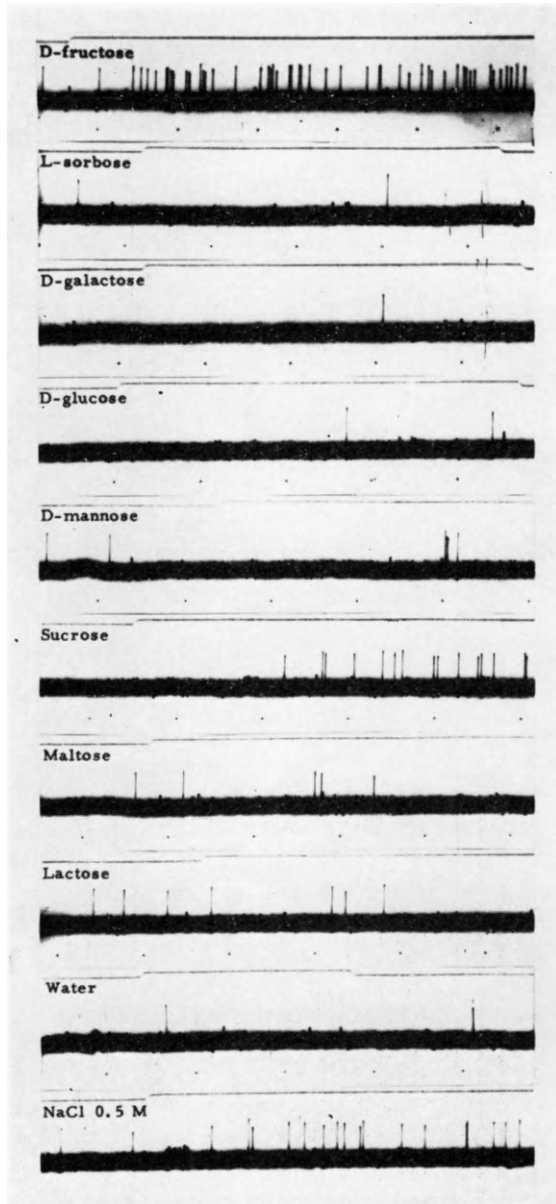
FIG. 7.   Records from an unspecific fibre from chorda tympani.   This fibre responded to salt as well as to sugar.   Time in seconds.

much the same, our analysis of the functional single fibres have revealed some detailed, new information.

It will be remembered that some of the fibres studied were activated by all of the sugars tested (Fig. 6). All of these fibres exhibited the largest responce to D-fructose and next to sucrose. However, we also noted fibres which displayed electrical activity only after stimulation with these two sugars. The sweet receptors, therefore, appear to have different threshold values, and consequently, D-fructose and sucrose elicit their large responses from the whole chorda tympani not only by activating the individual fibres to discharge with a higher frequency than the other sugars do, but also by activating more fibres.

The marked differences in stimulating ability displayed by the various sugars is certainly a result of differences in chemical and physical properties. One very interesting observation may be made by comparing the molecular configurations and sweet stimulating ability of the three disaccharides used, and those of the three monosaccharides which participate in the molecules of these disaccharides : Among the monosaccharides the stimulating ability decreases in the following order, D-fructose >D-glucose >D-galactose. The corresponding rating for the disaccharides is sucrose > maltose >lactose. In all of these three disaccharides D-glucose constitutes one half of the molecule, the other component being D-fructose, D-glucose and D-galactose, respectively (Fig. 1). Hence, the following rule appears to hold for the six sugars considered : Since all of the disaccharides have D-glucose in common as one half of the molecule, their relative sweetness and stimulating power may be determined by the second constituent monosaccharide, and in such a way that each of the disaccharides is less powerful in its sweet stimulating ability than an equimolar solution of that monosaccharide participating in its molecule which is *not* common to the other two disaccharides. For these six sugars we should consequently have the following rating of decreasing potency : D-fructose >sucrose >D-glucose >maltose >D-galactose >lactose. The discussion is summarized in the following scheme :

$$\text{D-fructose} > \text{D-glucose} > \text{D-galactose}$$

$$\text{Sucrose} > \text{Maltose} > \text{Lactose}$$
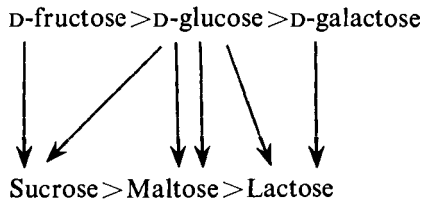
and

$$\text{D-fructose} > \text{sucrose} > \text{D-glucose} > \text{maltose} > \text{D-galactose} > \text{lactose}$$

These results indicate that the disaccharides are not split on the tongue or at the receptor sites when the stimulating of the proper receptors takes place.   If indeed an appreciable cleavage of the disaccharide molecules into the constituent monosaccharides had taken place, the actual number of the stimulating molecules would have been much increased over that of the solutions containing the pure monosaccharides.   Consequently, the solutions of sucrose, maltose and lactose would have been expected to yield a correspondingly larger response.
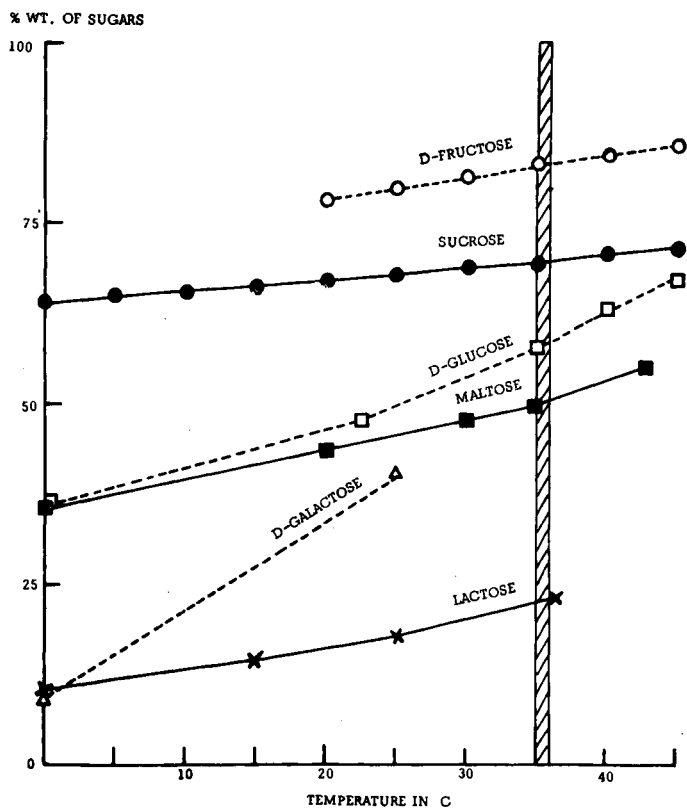

FIG. 8.   Water solubility of sugars studied. Cross-hatched bar indicates temperature of solutions when applied to the tongue. (After *International Critical Tables of Numerical Data, Physics, Chemistry and Technology*.   First Ed. Vol. II, Saccharimetry.   National Res. Council of the U.S.A. McGraw-Hill, New York 1926, and A. Seidell :   *Solubilities of Organic Compounds*.   D. Van Nostrand, New York 1941.)

At least one of the physical properites of the sugars used fits perfectly with the findings reported in this paper, namely, the water solubility.   The latter quantity has been furnished in Fig. 8, and a comparison between this figure and Table 1 reveals that greater water solubility is associated

with stronger stimulating power and sweeter taste. This certainly does not imply that sweetening power is a simple function of the water solubility ; but that the molecular characteristics responsible for water solubility also are important for the events which lead to stimulation of the proper receptors can hardly be circumvented. It has been hypothesized that sweetening power is often associated with low water solubility (Crozier, 1934, quoted by Pfaffmann, 1959), but in the case of the sugars this does not seem to be true.

B. The Peripheral Interaction between Salt and Sugar

1. *Responses of the whole chorda tympani.* The integrated responses of the whole chorda tympani to 0.5 M solutions of sucrose and sodium chloride, and to a mixture containing 0.5 M sucrose *and* 0.5 M sodium chloride are shown in Fig. 9. There was no water rinse between two successive applica-
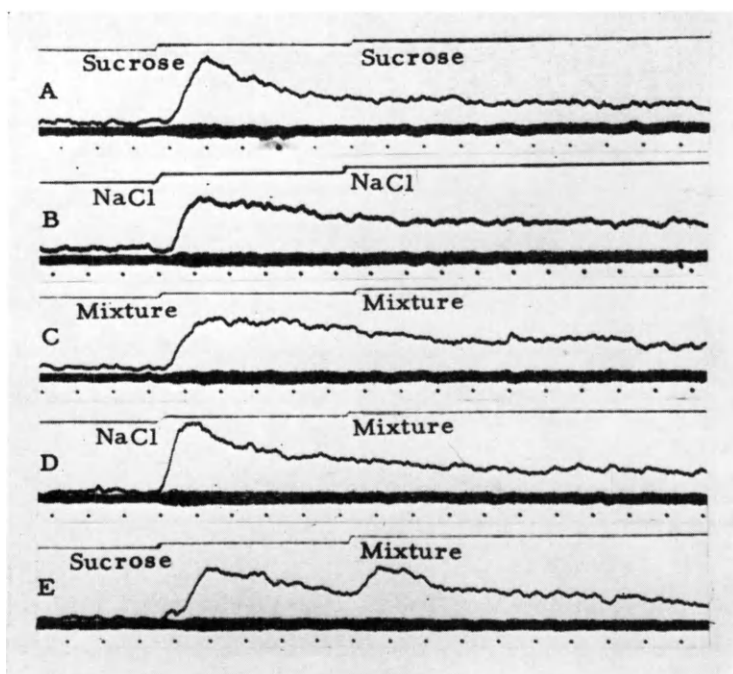
FIG. 9. Integrated responses recorded from the whole chorda tympani nerve. Note the depressing effect of sodium chloride on the response of the mixture in D. Time in seconds.

tions of the test-solutions. Figure 9 A, B and C are the control experiments in which the same solution was applied twice in succession. As seen, there was in no case a response to the second application. Figure 9 D shows that the mixture when applied after 0.5 M NaCl did not elicit any response,

whereas when it followed after 0.5 M sucrose, it produced a marked deflection of the integrated curve (Fig. 9E). Even a sodium chloride solution of only 0.2 M strength was able to strongly depress the response to a mixture containing 0.5 M sucrose *and* 0.2 M NaCl, whereas this mixture elicited a pronounced second response when it followed after the 0.5 M sucrose solution (Fig. 10B and D).
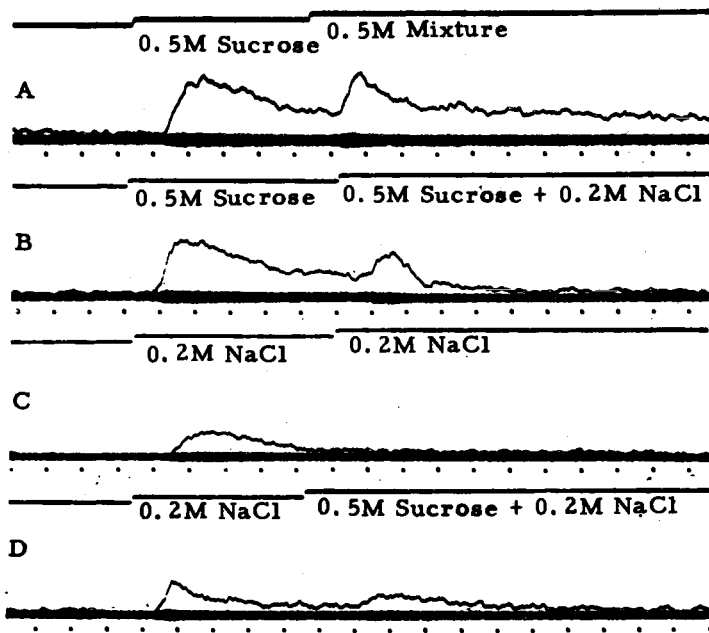

FIG. 10. Depressing effect of 0.2 M sodium chloride on the response to mixture containing 0.5 M sucrose and 0.2 M sodium chloride. Time in seconds.

2. *Responses of individual fibres.* The functional single fibres studied may be grouped into three classes, of which the second may be subdivided into three types, as shown :

1. Specific sweet fibres

2. Fibres responding to salt and sugar.
   - I. Sugar response > salt response.
   - II. Sugar response < salt response.
   - III. Sugar response = salt response.

3. Specific salt fibres.

(i) *Sweet fibres.* All of the fibres, by our criteria specific to sugar, were inhibited by previous application of 0.5 M sodium chloride to the tongue. This is illustrated in Fig. 11 where A shows the typical response of such a fibre to 0.5 M sucrose. In Fig. 11B the inhibition of the sucrose-response by

pre-treatment with 0.5 M NaCl is evident, but after a water-rinse, the normal response reappeared (Fig. 11C).

(ii) *Sugar-salt fibres.* The recording obtained from a type I fibre is shown in Fig. 12. The 0.5 M solution of sucrose elicited a conspicuous burst of electrical activity in the fibre when applied to the tongue, whereas the mix-
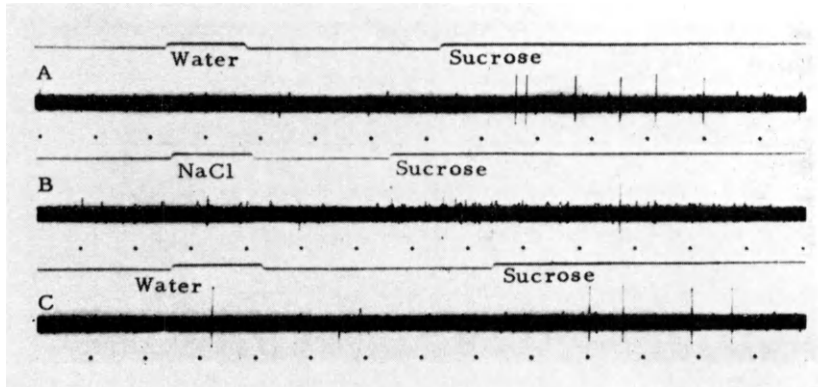

FIG. 11. Records from a single " sugar " fibre. Response to 0.5 M sucrose is inhibited by previous 0.5 M sodium chloride. Time in seconds.
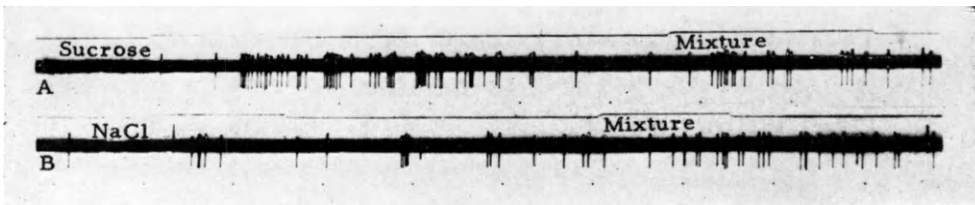

FIG. 12. Responses recorded from a single " sugar-salt " fibre (type I) upon application of 0.5 M mixture after 0.5 M sucrose (A) and 0.5 M sodium chloride (B). Time in seconds.

ture which followed produced a relatively small, but significant extra effect (Fig. 12A). The sodium chloride solution alone gave only a small effect in terms of electrical activity recorded from the fibre, but it depressed the response to sucrose markedly as seen from the relatively modest response to the mixture which followed (Fig. 12B).

An example of the response pattern of a type II fibre is furnished in Fig. 13. Sucrose gave a very small response in this preparation, whereas the sodium chloride had a marked ability to stimulate. In accordance with these characteristics mixture after sucrose activated the fibre conspicuously (Fig. 13A), while mixture after sodium chloride had only a relatively slight additional effect (Fig. 13B).

The peripheral depression of the sugar response by salt was clearest seen

in the recordings obtained from the type III fibre. Two figures are furnished in order to illustrate this phenomenon. In Fig 14A the response to
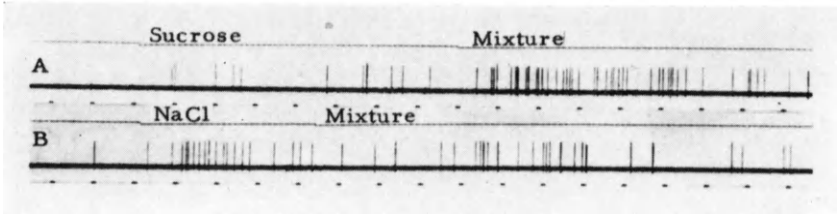sucrose after a water rinsing is seen to be a conspicuous burst of spikes, and a

FIG. 13. Responses recorded from a single " salt-sugar " fibre (type II) upon
application of 0.5 M mixture after 0.5 M sucrose (A) and 0.5 M sodium chloride (B).
Time in seconds.
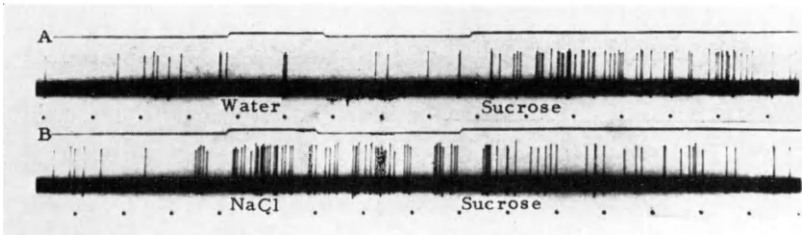
FIG. 14. Responses recorded from a single fibre of type III.   Note the response
to 0.5 M sucrose is depressed by previous 0.5 M sodium chloride.   Time in seconds.
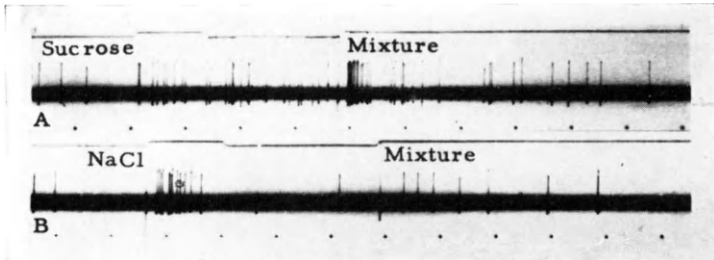
FIG. 15. Records from a single fibre of type III showing the depressing effect
of sodium chloride on the response to mixture. Time in seconds.

similar response is elicited from the fibre when stimulated with sodium
chloride (Fig. 14B).   However, when sucrose followed after an application
of 0.5 M NaCl without intermediate rinse with water the response to the
sugar was very much less than before.   Again, in Fig 15A it is shown that
the electrical activity in a type III fibre to the mixture was very marked
when the tongue had been flushed with 0.5 M sucrose solution just before,
whereas treatment with sodium chloride prior to the application of the
mixture nearly abolished the response from the fibre.

(iii) *Salt fibres.* The fibres which, by our criteria, were specific for salt stimulation were never affected by previous application of 0.5 M sucrose to the tongue. This is evident from Fig. 16A and B, in which the responses of the fibre to 0.5 M NaCl after water and sucrose, respectively, are reproduced.
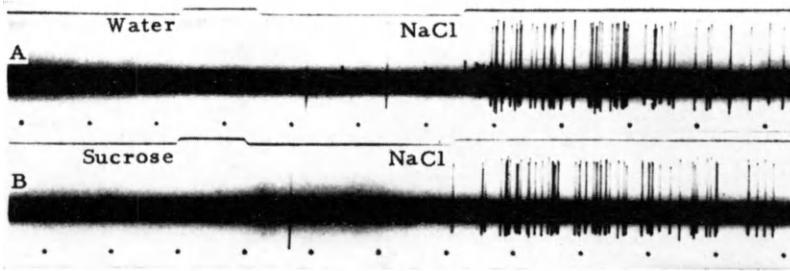
FIG. 16. Records from a single " salt " fibre showing that the response to 0.5 M sodium chloride is not affected by previous application of water (A) and 0.5 M sucrose (B). Time in seconds.

The data presented in this section has been summarized in Fig. 17. This schematic review of the electrical discharges from the various types of functional single fibres studied explains readily the response patterns of the integrated recordings which are shown at the bottom of the figure.

The mechanism by which the salt solution exerts its inhibitory effect on the sugar response is, however, not as easily revealed. As a matter of fact,
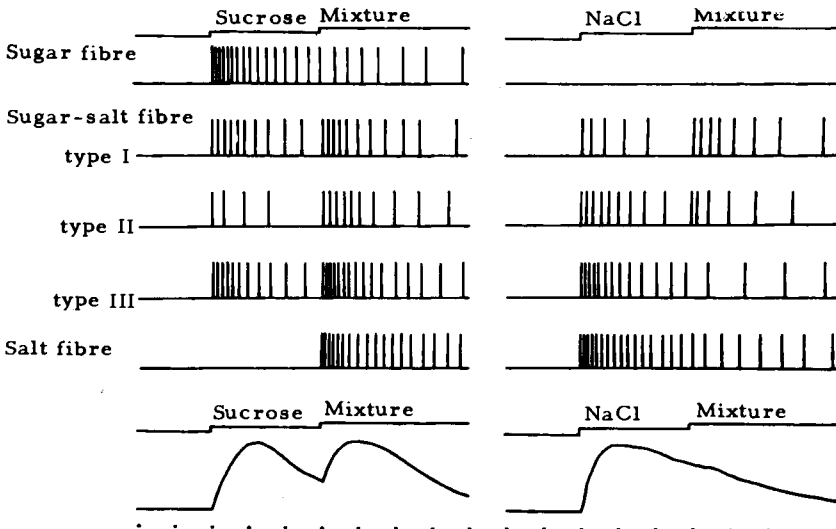
FIG. 17. Schematical representation of response patterns of various types of fibres and integrated responses to salt and sugar solutions.

we are at the present time left without any experimental clues to the solution of this problem.   There is one hypothetical possibility, however, which may not be too far-fetched, namely, that a heavy absorption or electrostatic binding of salt takes place on the surface of the taste receptors so that the sugar molecules simply are prevented from making contact with the proper receptor sites.

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ELECTROPHYSIOLOGICAL STUDIES ON HUMAN TASTE NERVES

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The first attempts to record the electrical gustatory response of man were
made in order to solve the problem whether man, like the monkey and
many other species, has gustatory fibres responding positively to water.
By a freak of nature the gustatory fibres from the anterior tongue separate
from the lingual nerve trunk and run through a bone channel into the
middle ear, where it is often exposed during otological operations. The
first experiments to place leads on the chorda tympani during middle ear
operations were made during the years 1956–57 in Södersjukhuset, Stock-
holm by Åhlander and one of us (Y.Z.). Out of ten trials in which the
surgeon applied the electrodes to the exposed chorda tympani in the cavum
tympani, we obtained very weak signals in only two cases in response to
cold and gustatory stimulation of the tongue. However, the responses were
just audible in the loudspeaker and could not be recorded.

In 1958 successful experiments were performed in the Ear Clinic of
Karolinska Sjukhuset, Stockholm, during operations undertaken in an
effort to mobilize the stapes (Diamant and Zotterman, 1959). In these
experiments we recorded the integrated electrical response of the nerve
to touch and to various taste solutions. As will be seen from Fig. 1
there was a good response to 0.5 M NaCl solution, 15 per cent sucrose,
0.04 per cent saccharine, 0.02 M quinine sulphate and 0.2 M acetic acid.
However, the application of water to the tongue was followed by a reduc-
tion in the spontaneous activity in the nerve in exactly the same fashion as
we had previously found in the rat, which does not possess any taste
fibres that respond positively to water.

This report presents the results of further experiments on man giving
more detailed information about nerve responses to various sapid sub-
stances.

TECHNIQUE AND PROCEDURE

Experiments were carried out on 32 otosclerotic cases undergoing a

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middle ear operation. The operations were performed in the ear clinic of Karolinska Sjukhuset in an electrically screened operating room ; the 50 c/s power supply to this room was switched off during recording. The patient was earthed by applying a metal net collar around the neck as well as a metal cuff to the arm used for intravenous injections and for blood
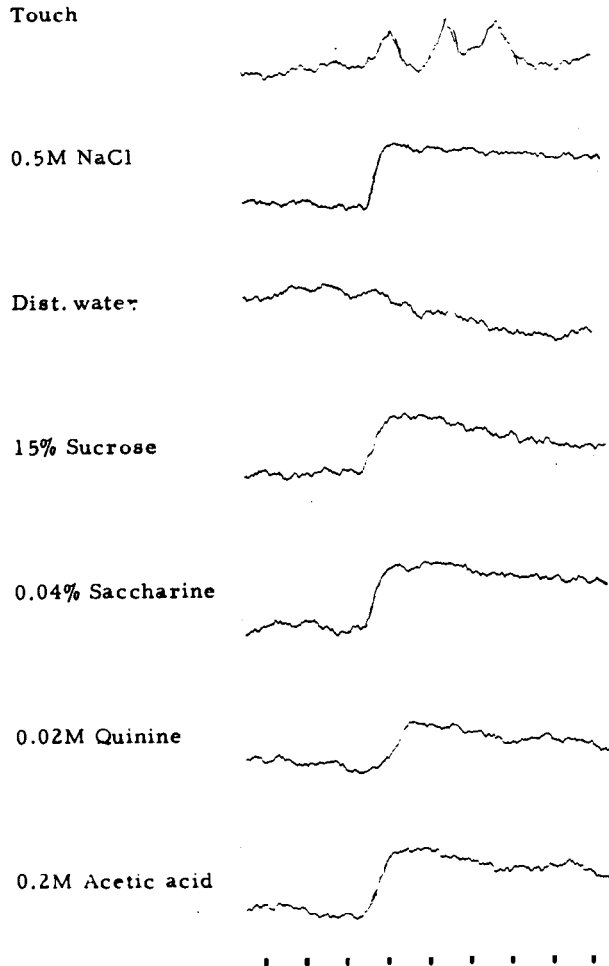
FIG. 1. Integrated electrical responses of the whole chorda tympani of man to sapid solutions poured on the tongue. Note that water depressed the spontaneous afferent activity. Time in seconds.

pressure measurements. The experiments were performed under deep anaesthesia (Fluothan) and in some cases the patient was curarized in order to avoid movements of the electrodes.

After an incision in the meatus, the ear drum was pushed aside giving

free access to the cavum tympani. In the majority of cases it was fairly easy to dissect the chorda tympani free from the surrounding bony structures but in some cases the chorda was completely covered by bone and had to be ground free. In general, such cases gave poor results as the chorda tympani has a more gelatinous structure when inside a bony channel than when it is outside and surrounded by connective tissue. For that reason it also proved futile to split up the nerve into small strands for single fibre recordings. Bleeding during the operation was stopped by applying pure adrenaline solution without any anaesthetic addition. The chorda was cut centrally as near as possible to the point where it comes out from the 7th nerve canal. The sheath was removed leaving a naked nerve trunk for a length of 3–5 mm which could be put on the electrode.

In the beginning we used a double platinum electrode which was fixed on the ear speculum but later we found it more convenient to use a single electrode. The electrical response was recorded by means of an r.c.-coupled amplifier and a two channel tape recorder (Tandberg stereo). Thus the electrical response from the nerve, the signals and the minutes of the experiment were simultaneously recorded. After the actual experiment the tape records were processed in the laboratory by means of an integrator and a cathode ray oscillograph or an ink-writer (Mingograf) producing the integrated records of the electrical responses.

Sapid substances were applied to the tongue by means of a special dispensing device fixed in position with its tip approximately 3 mm above the tongue. To the stopcock a switch was attached which gave a signal on the record when the test solution (15 ml at 30°C) was released. The tongue as well as the burette was rinsed with 30°C water before each trial.

<div align="center">RESULTS</div>

In eight out of 32 patients tested we did not obtain any response whatever from the chorda tympani. In another seven cases the response was very poor or electrical disturbances occurred. Thus there remained 17 cases in which the records were good enough to be analyzed further.

As is the case in other mammals, the human chorda responded to mechanical and cold stimulation of the tongue, and the mechanical response was comparatively weak, which implies that only a few mechanoceptive fibres run via the chorda tympani. Warm water 37–43°C generally gave no response while cold water gave very pronounced responses. None of the chordas tested responded positively to the application of distilled water of the same temperature as the surface of the tongue.

Response to NaCl

Positive responses to NaCl solutions were obtained in all the successful experiments and in six cases we obtained a complete series of responses to

NaCl in solutions from 0.001 to 1.0 M. When the peak amplitudes of the integrated responses were plotted against the logarithm of the molarity we obtained in all six cases a fairly straight curve up to 0.2 M NaCl solution.

FIG. 2. Graphs showing the relation between peak height of electrical response and the molarity of the NaCl solution applied to the tongue.
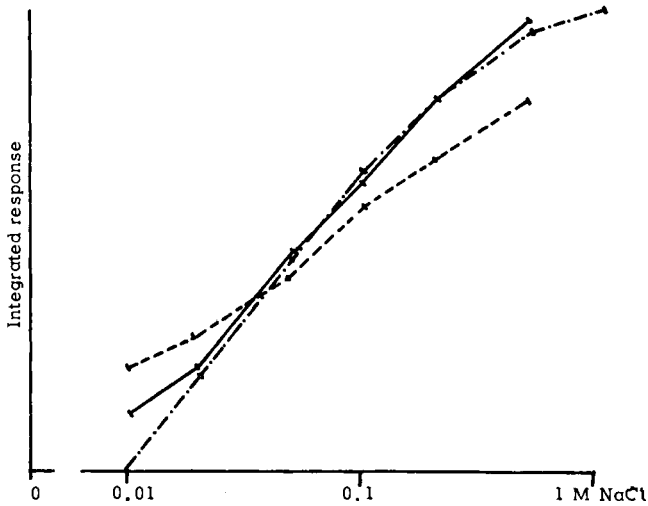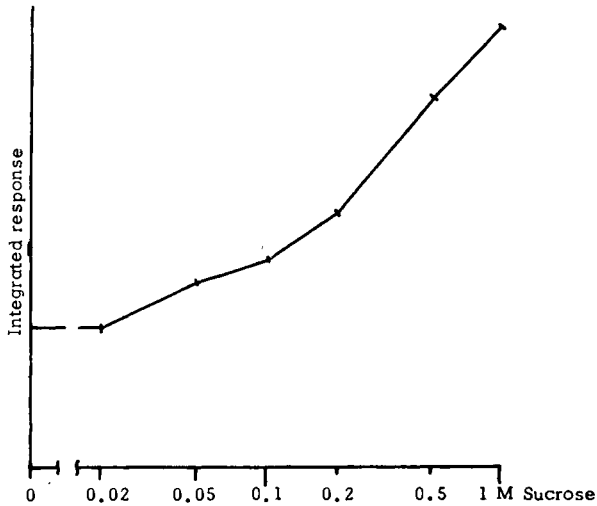

FIG. 3. Graph showing the relation between peak height of response and the molarity of the sucrose solution applied to the tongue.

After this point the curve tends to level off (Fig. 2). The threshold seems to be around 0.01 M NaCl which is in good accordance with the values obtained in psychophysical experiments on man (v. Skramlik, 1926).

Responses to Sugars

All responding preparations of the human chorda tympani reacted to sugars and also to saccharin. The threshold concentration of cane sugar lies just above 0.02 M in accordance with the threshold of 0.017 M obtained by Heymans in psychophysical experiments on man (see v. Skramlik, 1926). The response-molarity curve shows a rather straight line up to a concentration of 1 M sucrose (Fig. 3).

The effects of different biological sugars were recorded in two cases (Fig. 4). From the diagrams in Fig. 5 it will be seen that sucrose produced
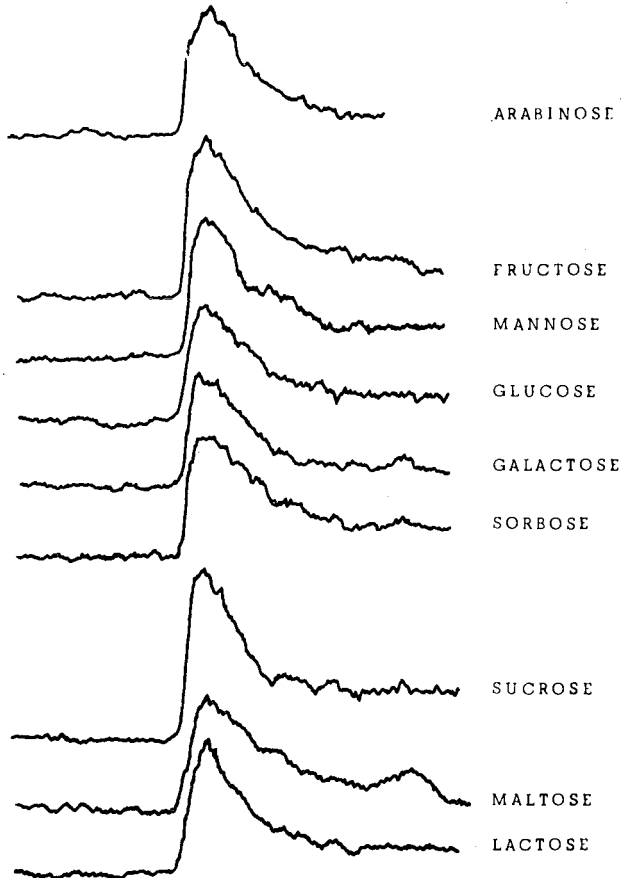
FIG. 4. Integrated records from the human chorda tympani in response to various solutions of biological sugars.

the biggest response, followed by that of fructose, mannose (which tastes bitter), glucose and maltose, sorbose and arabinose and finally galactose. The response of lactose was unexpectedly high (77 per cent of sucrose). Except for this relative high value, the recorded heights of the integrated

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responses are in good accordance with the relative sweetness of sugars obtained in experiments on man (see v. Skramlik, 1926 ; Andersen and Zotterman, 1962).

Alcohol

We have only one series of alcohol tests on the chorda tympani of man. The integrated response of such a series (Fig. 6) shows that alcohol from 0.5 to 2 M produced a small and rapidly declining response. At concentrations above 3 M, the alcohol elicits not only a considerably stronger initial

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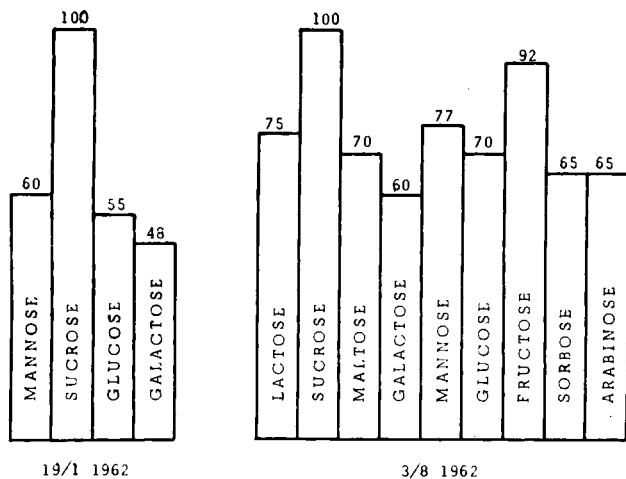
FIG. 5. Diagrams showing the relative peak height of the electrical response of the chorda tympani in two subjects to the application of various sugars to the tongue.
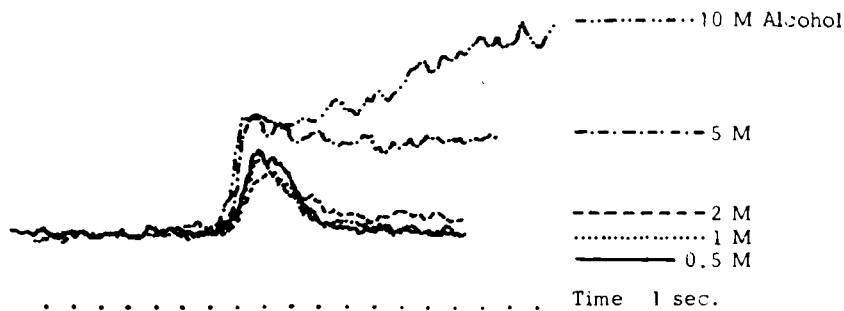
FIG. 6. Integrated responses of the human chorda tympani to the application to the tongue of alcohol solutions of varying molarity. Time in seconds.

response but also a long lasting afterdischarge. This afterdischarge slowly increases to levels even higher than the initial peak value of the integrated response (Fig. 6).

For comparison, these alcohol experiments were repeated on the dog's tongue. Figure 7 shows the integrated response from the dog's chorda tympani to increasing concentrations of alcohol followed by a water rinse. From these records it will be seen that there is no positive response until a 3 M solution was applied. As will be seen from Fig. 8 the response comes slowly after a relatively long latency. At 5 M concentration the latency is
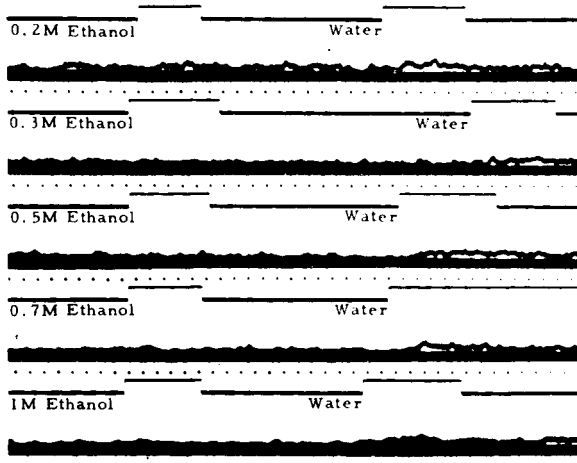
FIG. 7. Integrated responses of the dog's chorda tympani to the application of weak alcohol solutions to the tongue. Note the absence of response to the weak alcohol solutions while water gave weak positive response. Time in seconds.
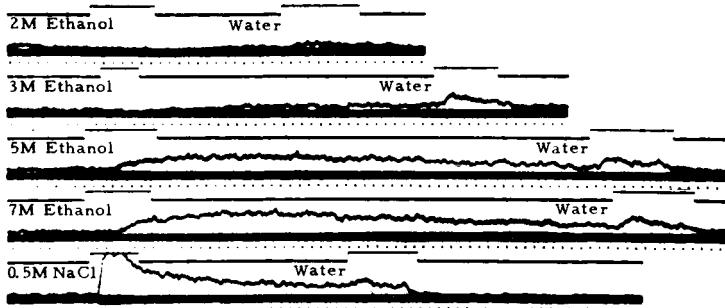
FIG. 8. Integrated responses of dog's chorda tympani to the application of strong alcohol solutions to the tongue. Note the marked depressive effect of the 2 and 3 M solutions before the onset of the slowly rising positive response. Time in seconds.

shorter and the discharge reaches its highest values after about 8 sec and declines quite slowly. A 7 M solution gives a stronger but otherwise very similar response.

It is interesting to note that the alcohol even at as low concentration as 0.2 M obviously depressed the positive response to water. The immediate depressive effect of alcohol is still more marked at 2 M and 3 M concentrations before the slow onset of the positive response (Fig. 8). The response to the subsequent application of water was only slightly stronger after higher alcohol concentration than after the weaker solutions.

In order to obtain more detailed data, the chorda tympani of the dog was split into fine strands. We thus obtained one preparation which responded
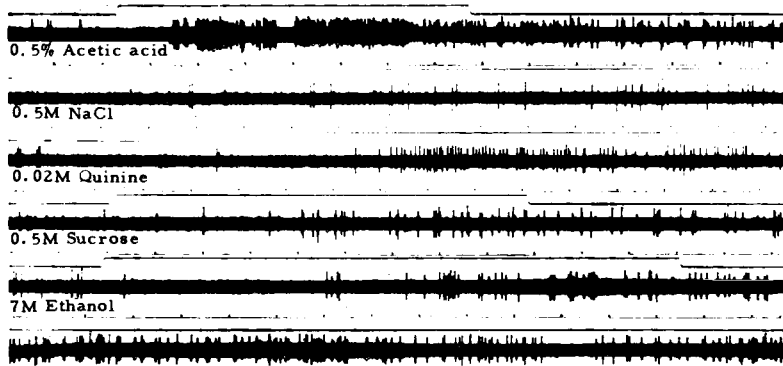
FIG. 9. Records from a fine strand of the dog's chorda tympani. Note the responses to acid and quinine and the weak response to sucrose and alcohol. Time in seconds.

to acid and quinine but only weakly to sucrose and not to salt (Fig. 9), and another preparation which showed a massive response to sucrose but not to acid, salt or quinine (Fig. 10). The response to alcohol was positive in both cases after a 4 to 6 sec latency. It was, however, much more pronounced in the strand containing fibres strongly responding to sucrose.

A similar series of alcohol tests were made on 13 subjects who reported the taste of alcohol solutions poured on their tongue. The reports were very varied. As will be seen from the diagram of Fig. 11 the gustatory threshold varied but the median value was 0.5 M. This was thus the threshold concentration of alcohol at which they felt a definite taste. This taste sensation varied between sweet, sour and bitter. None reported salt except at much higher concentrations. Seven subjects reported sweet taste while the remaining six experienced no sweet sensation at any concentration. Bitter taste was reported by all 13 subjects at a threshold median value of 0.8 M. Sour taste was reported by six subjects at various concentrations. Only three subjects reported a salty taste with thresholds 0.7, 2 and 3 M respectively. All subjects but one felt a smarting or burning sensation at concentrations above 3 M.

DISCUSSION

We have only been able to record the electrical response from the whole nerve trunk of the tympanic part of the chorda tympani of man.   All our attempts to split the nerve into fine strands have failed.   We believe that
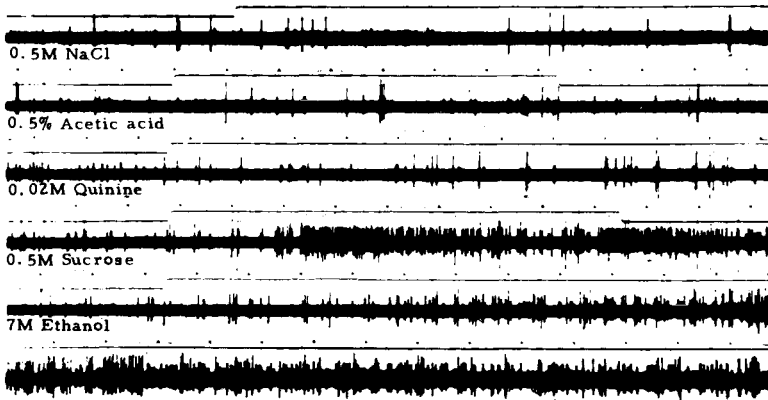
FIG. 10.   Records from a fine strand of the dog's chorda tympani which responded massively to sucrose but not to acid, salt or quinine.   Note the much stronger response to alcohol from this preparation compared to the response to alcohol of the nerve strand recorded in Fig. 9.   Time in seconds.
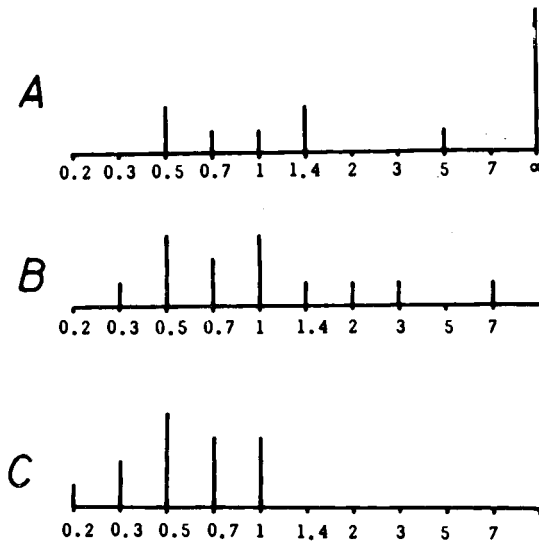
FIG. 11.   Histograms showing the distribution of 13 subjects according to their threshold sensation of A sweet and B bitter.   C shows the distribution of thresholds for the discrimination of alcohol solutions against water.

this is mainly due to the physical properties of the chorda tympani in the middle ear.   It seems as if peripheral nerves lose a lot of their connective

tissue in the sheath and particularly between the individual fibres when they are situated in a bony canal. This connective tissue makes the nerve mechanically more resistant as it passes between muscles or other movable tissues. Our analyses of the gustatory activity has thus been limited to the study of the integrated response from the entire nerve.

Even if this restricted our analyses, we have nevertheless obtained a few answers to hitherto unsolved questions. Thus it is obvious that water has no positive action on the gustatory apparatus of man contrary to the case in the rhesus monkey and in the dog and many other mammals except the rat.

It is of special interest to compare the thresholds as well as the relative gustatory values of different sapid solutions obtained from human chorda tympani records, with the subjective values collected from psychophysical experiments. The close correlation between the threshold values obtained by these different methods is interesting as it shows that even the weakest signalling registered in the taste fibres is communicated to those parts of the central nervous system which are responsible for conscious perception.

Although our electrophysiological data are limited to only one case of each species it is interesting to note the difference between responses to alcohol in man and dog. The dog's tongue possesses gustatory receptors for substances that taste sweet in man with the exception of saccharin. It must be remembered that the dog has gustatory receptors responding to water. Thus the absence of response in the dog to weak alcohol solutions (0.5 to 2 M) must be due to a depressing action of alcohol on the receptors responding to water. The delayed response to alcohol in the dog's chorda seems to coincide in its temporal course with the afterdischarge to strong alcohol solutions in the human chorda.

From our experiments on the dog's chorda it looks as if alcohol preferentially stimulated gustatory nerve fibres which respond to sucrose. Since 7 out of 13 of our human subjects reported the alcohol sensation as sweet, it seems likely that the human tongue possesses similar gustatory fibres responding to sweet tasting substances as those described in the dog (Andersson et al. 1950). At higher alcohol concentrations all gustatory nerve fibres may be stimulated in addition to a strong stimulation of trigeminal nociceptive fibres before the endorgans are paralyzed by the strong alcohol which, of course, belongs to the group of substances which when locally applied produce an anesthesia dolorosa.

SUMMARY

1. Integrated electrical responses to sapid substances have been recorded from the tympanic part of the chorda tympani of man during otological operations.

2. The human chorda responds to the application to the tongue of solutions of different sugars and saccharin, quinine, acids and salts. We did not obtain any positive gustatory response to water in man contrary to what is the case in the rhesus monkey and some other mammals other than the rat.

3. There is a close correlation between the weakest electrical response from the chorda and the threshold for salt and sucrose obtained in psychophysical experiments.

4. Alcohol on the tongue produces an electrical response from the chorda tympani already at 0.2 M concentration. Above 3 M concentration alcohol produces in man a long lasting afterdischarge. This afterdischarge coincides in its time course with a burning sensation. The initial phasic response to alcohol of low concentrations recorded from the chorda seems to derive from gustatory fibres, some of them responding preferentially to sweet tasting substances. At increasing concentrations alcohol stimulates gradually all kinds of afferent fibres in the chorda before at high concentrations the alcohol finally produces a local anesthesia.

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SENSORY NEURAL PATTERNS AND GUSTATION*

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AT present there is no thorough understanding of the nature of the afferent neural message for taste quality.† This is due in large part to our lack of knowledge about the significant aspects of the taste stimulus. However, it is possible to come to a partial analysis of the neural message *without* this knowledge about the stimulus. The nature of this analysis will be made clear by reference to those sensory systems where the neural message has been worked out in some detail.

METHOD TO DETERMINE NUMBER OF SENSORY FIBER TYPES

In color vision there are probably very few receptor types, these being represented in the optic nerve by a corresponding number of fiber types.‡ Assume that there are only three such receptor-fiber types, and let the three curves in Fig. 1A represent the responsiveness of these three types to the various wavelengths of light. Since in taste the significant parameters of the stimulus are not understood fully (as pH, etc.), let us suppose that in vision also the nature of the stimulus for color is not understood. Assume that four colors are available as stimuli and recordings are obtained from a series of single optic nerve fibers using first the stimulus indicated as Q in the figure. What kind of recordings will result ? It is seen from the ordinate erected at Q that the amount of neural activity that would be recorded may take only three values depending on which type of optic nerve fiber was being sampled. If the recording was from fiber type 1, the value that would be obtained is indicated by the intersection of this ordinate and the 1 curve ; similarly, for fiber types 2 and 3, the amount of activity that would be obtained is represented by the values of curves 2 and 3 at the Q ordinate. *Since there are only three fiber types in this*

† " Taste quality " refers to the " salt-sour-sweet-etc." aspect of taste as distinct from intensity.
‡ Although perhaps inexact, the assumption is made in this paper that the stimulus sensitivity functions of receptors are reflected without change in their lower-order afferent neurons, and that arguments referring to one apply also to the other.
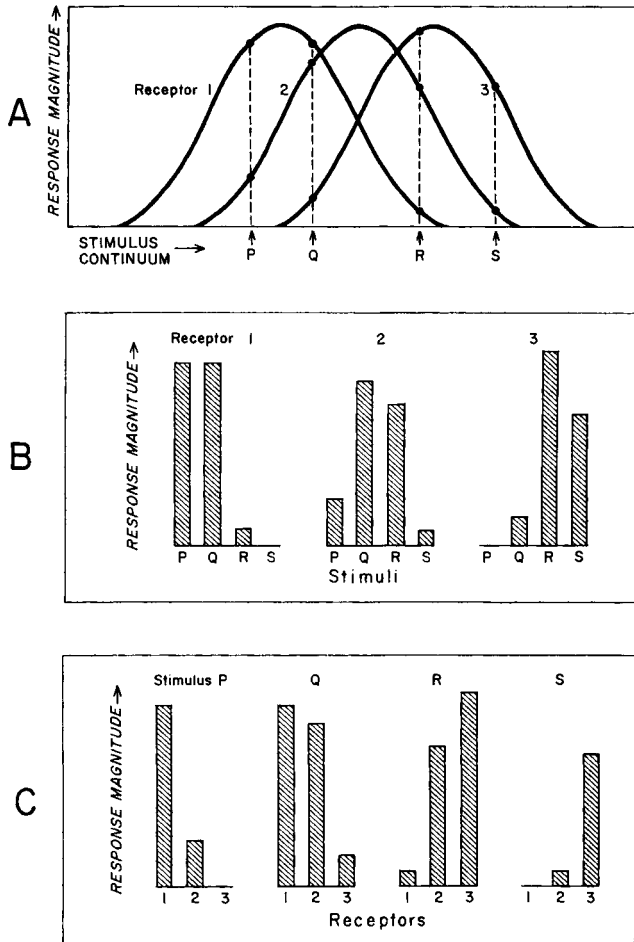
FIG. 1.   Afferent fiber types and patterns of neural activity.

A.   Afferent fiber-types (or receptor types).   Curves 1, 2, and 3 represent the responsiveness of three hypothetical afferent fiber types (or receptor types) along a hypothetical stimulus continuum. P, Q, R, and S represent four stimuli along this stimulus continuum.   The responsiveness of a fiber type to one of these stimuli is indicated by the intersection of the response curve and the ordinate erected at the stimulus.

B.   Responsiveness of the three fiber types to the four stimuli in A.   In each of the bar graphs is shown the responsiveness of one of the fiber types to each of the stimuli in A.   If recordings were obtained from one of the fiber types shown in A using these stimuli, one of these three " response profiles " would be obtained, depending upon which fiber type was being sampled.   There would be as many " response profiles " as fiber types.

C.   Across-fiber patterns.   In these bar graphs are shown the patterns of activity across the three fiber types produced by the four stimuli in A.   Each stimulus produces a characteristic pattern across the three fiber types.   There would be as many across-fiber patterns as stimuli.

example, only three response magnitudes (including zero) could result from stimulation with any given color. The three values which would be obtained with stimulus Q are indicated in the bar graphs in Fig. 1B. In these three bar graphs are represented the activity which would be recorded from fiber types 1, 2 and 3. In each of these three graphs, the bar for Q indicates the amount of neural activity that would be recorded from that fiber type with the stimulus Q. Similarly, stimulus P (or R, or S) would evoke characteristic response magnitudes in each of these fiber types as indicated. Whenever we record from fiber 1, then, using these four stimuli, we will get a bar graph with the " profile " indicated under 1. Similarly, fiber types 2 and 3 will yield characteristic profiles. If a large number of optic nerve fibers are sampled using these four stimuli, one of these three bar graph profiles will be obtained from each fiber. *Since only three " response profiles " could be derived from these fibers, the conclusion would be reached that there are only three fiber types. Note that this conclusion could be reached without having knowledge of the relevant parameter of the stimulus (wavelength), and without being able to vary the stimulus systematically.*

The same kind of analysis could be accomplished in audition demonstrating the effect of a multiplicity of fiber types. Again assume that the significant aspect of the stimulus for pitch is not understood, but four tones are available as stimuli. If these many fiber types were represented in Fig. 1 there would be very many curves in Fig. 1A, and a corresponding number of bar graphs in Fig. 1B. *It would be concluded from the number of bar graph profiles of responses obtained from auditory neurons that there were very many fiber types, even if we did not understand the nature of the stimulus, and did not vary it systematically.*

DETERMINATION OF THE NUMBER OF
GUSTATORY FIBER TYPES

The above considerations give us some clues concerning the sensory neural message for taste quality. Many investigators have collected the type of bar graph for taste shown in Fig. 1B. Some examples are shown in Figs. 2 and 3. Those in Fig. 2 were obtained from single second-order neurons in the nucleus of the solitary tract of rats anesthetized with pentobarbital sodium. KCl-filled glass micropipettes were used. Similar bar graphs describing the responsiveness of first-order neurons in the chorda tympani nerve of the rat are shown in Fig. 3. With reference to Fig. 1B, it would not be easy to place these graphs into a few groups on the basis of the profiles they present, but rather there would be very many groups. (It is, of course, not necessary that these groups be represented by smooth, uninterrupted curves on uninterrupted continua ; e.g., it would be

impossible to devise a strictly continuous baseline out of a series of diverse anions. These could, at best, designate points on a discontinuous baseline.) Therefore, the first conclusion that can be drawn about the nature of the sensory neural message for taste quality is that *there are many fiber types representing gustatory quality* as for pitch discrimination, rather than a few fiber types as in color vision.

MODEL AND METHOD TO DETERMINE SIGNIFICANT
ASPECT OF AFFERENT MESSAGE

How might the fiber types in taste be classified? Let receptor 3 in Fig. 1 now be labeled a " red " receptor. This is a somewhat misleading label because, although it has its maximum sensitivity in the red region of the spectrum, it responds to a wide range of stimuli, and in concert with the other receptor types, is probably responsible for signaling many
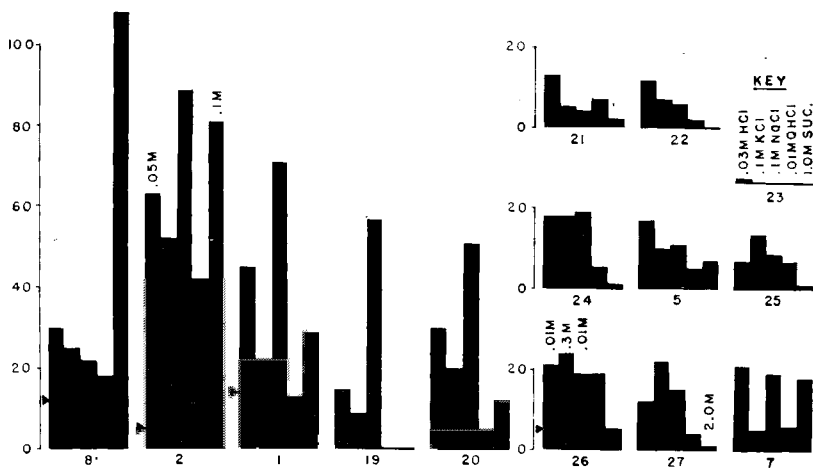
FIG. 2. Receptor profiles analogous to those shown in Fig. 1 B. Recordings obtained from single neurons in the nucleus of the solitary tract of the rat. Bar heights indicate number of impulses recorded in the first second of evoked activity. Small triangles indicate spontaneous level of activity.

wavelengths. To label a taste fiber a " salt " fiber because it is maximally sensitive to salts likewise may be misleading because it, as the red receptor, is probably responsible for signaling a number of stimuli. Actually, until the stimulus dimensions for taste quality are established, a fiber's point of maximum sensitivity cannot readily be established. Because of the possibility of a very large number of fiber " groups " as in audition, it is incorrect to categorize these fibers in a few groups unless such groups are established as indicated in Fig. 1B.

Since it is difficult to give meaningful labels to individual fibers or to define fiber types, how may this afferent neural message best be viewed? Pfaffmann (1) has suggested that, in taste, the afferent neural message for quality is probably expressed in terms of the relative amounts of
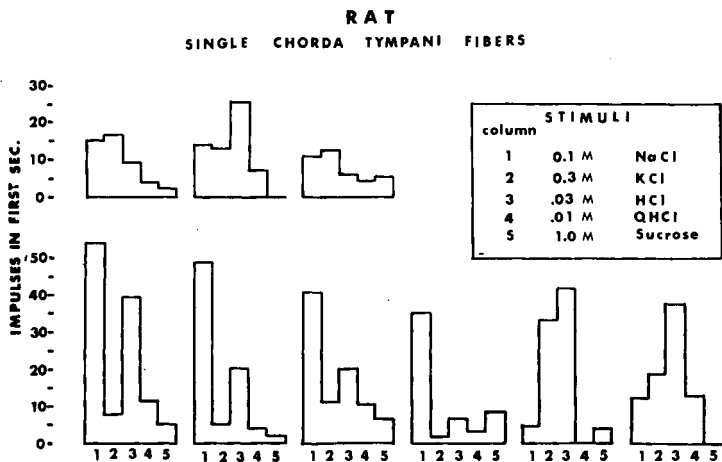
FIG. 3.   As Fig. 2 except recordings obtained from single chorda tympani fibers.

neural activity across many neurons.   The present thesis is that it would be more productive to consider the problem in terms of such across-fiber patterns than in terms of many receptor types.   It should be pointed out that in vision and audition, for example, the color or pitch of the stimulus is probably signaled by a pattern of activity in a number of fiber groups rather than by the activity in any single fiber group alone.   Bar graphs which show the across-fiber patterns of activity resulting in fiber groups 1, 2 and 3 from stimulation with a single stimulus (P, or Q, or R, or S) are presented in Fig. 1C.   For example, let Q represent a green stimulus. Whenever this stimulus is presented (Fig 1A), all fibers in group 1 respond to the extent shown by the intersection of curve 1 with the ordinate at Q, all fibers in group 2 respond to the extent shown by the intersection of curve 2 and this ordinate, and similarly for all fibers in group 3.   The resulting across-fiber patterning is given in Fig. 1C, stimulus Q.   These three levels of activity in these fiber groups would form a pattern to signal the stimulus Q, or green.   Presumably this kind of pattern signals the quality of the stimulus in vision and audition.

Can evidence be obtained that in gustation too, the significant aspect of the neural message is actually the pattern of activity across many taste neurons ?   There are two steps to the resolution of this question, one physiological and the other behavioral.   First, the across-fiber

patterns of response for a number of taste solutions must be observed. Second, it must be determined if these patterns are actually significant for the interpretation of the qualities of the taste stimuli. In the experiments which follow, neural patterns were determined for several taste substances ; predictions concerning the discriminability of these taste solutions were then made from their neural patterns and tested in behavioral experiments.

DETERMINATION OF SIGNIFICANT ASPECT OF AFFERENT MESSAGE; PHYSIOLOGICAL AND BEHAVIORAL

First, the responsiveness of a number of chorda tympani fibers to several taste solutions was determined in the rat anesthetized with pentobarbital sodium. Some of these data are presented in Fig. 4 in a way
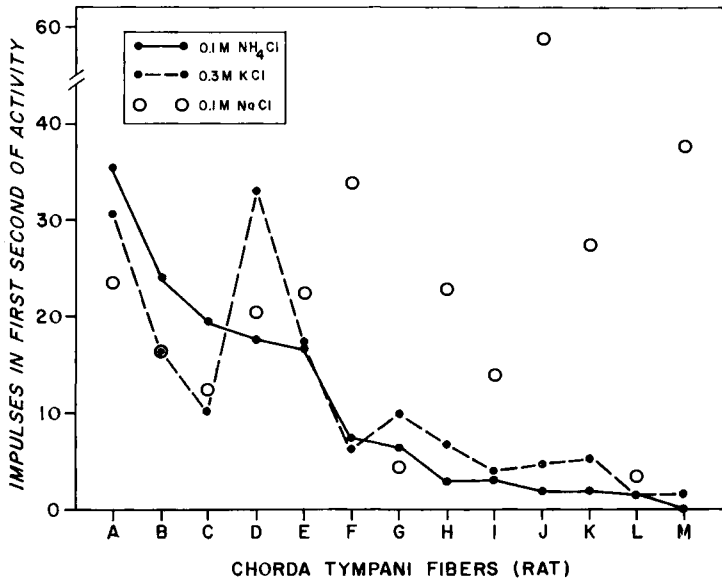
FIG. 4. Across-fiber patterns analogous to those shown in Fig. 1C, except that connected points are used instead of bars, and 13 fibres are shown instead of three. Single rat chorda tympani fibers. Fibers arranged along baseline in order of responsiveness to NH$_4$Cl. KCl pattern similar to NH$_4$Cl pattern. Neither of these patterns similar to the NaCl pattern.

which shows the across-fiber pattern. Here the fibers are arranged along the base line in order of their responsiveness to NH$_4$Cl. Any other arrangement would be satisfactory for the present purposes. The degree of their responsiveness to this solution is indicated by the black dots connected with the solid lines. In this way is shown a pattern of the amount of neural activity across many neural units, and is the across-fiber NH$_4$Cl

pattern.* According to the present hypothesis this pattern is what signals the quality of NH_4Cl at least as far as these 13 fibers are concerned.

A very important test of the pattern theory of taste quality sensitivity is that if the quality of a stimulus is actually signaled by this kind of patterning, then stimuli which give similar patterns should taste somewhat alike. Conversely, stimuli producing highly dissimilar patterns should taste considerably different. In Fig. 4 we see that KCl produces a pattern quite similar to that produced by NH_4Cl, at least as contrasted with the NaCl pattern. A measure of the similarity of these patterns is given by the correlation between the amplitudes of the responses produced by these solutions in these fibers. The product-moment correlation coefficient between the amplitudes of responses to NH_4Cl and KCl for these 13 fibers is $+0.83$, indicating a close similarity between these patterns. The pattern produced by NaCl, as indicated by the unconnected open circles in Fig. 4, is not very similar to either the KCl or NH_4Cl patterns. In Table 1 are shown correlation coefficients based on a larger number of fibers. Thus, the neural patterns predict that for the rat, KCl and NH_4Cl should have similar tastes, but neither of them should taste very much like NaCl.

(It is clear from Table 1, in which are presented correlations between the amount of response of the fibers tested to a number of taste solutions, that NH_4Cl, KCl *and* $CaCl_2$ all produce similar neural patterns, and that LiCl produces a neural pattern very similar to that produced by NaCl. High correlations also suggest that these stimuli are relatively adjacent on the stimulus continua. However, it can be demonstrated that low correlations indicate little more than that the neural patterns produced are dissimilar ; the relative placement of these pairs of stimuli on the stimulus continua are indicated more by the *form* of the correlation scattergram than by the degree of correlation.)

Now let us turn to the behavior predicted from the neural data. Two stimuli may be defined as perceptually similar to the extent that a response learned to one of them will generalize to the other. Thus, if these neural patterns are the basis for taste quality sensitivity, a response learned to one stimulus should generalize to another stimulus producing a similar neural pattern, but not as much to one giving a different pattern. If such predictions are borne out, it would be indicated that such neural patterns actually are the basis of taste quality sensitivity.

Two behavioral tests were employed, both based on the generalization of shock-based avoidance of drinking from one salt to others. In the first test, three rats each learned to avoid drinking one of three salts, KCl, NH_4Cl

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* Some other aspect of the across-fiber pattern of neural activity more subtle than number of impulses in the first second of evoked activity may prove more predictive of behavior.

or NaCl in a single tube test (total of nine rats). Shocks (1 mA for 0.3 sec) were delivered through the feet whenever the rat drank for about 2 sec. On several days following the avoidance training, recovery of drinking

TABLE 1.   CORRELATIONS BETWEEN SINGLE CHORDA TYMPANI FIBER RESPONSES TO A NUMBER OF TASTE SOLUTIONS. ONLY ONE CONCENTRATION OF EACH SOLUTION USED. IN EACH CELL IS GIVEN FIRST THE PRODUCT-MOMENT CORRELATION COEFFICIENT, THEN THE NUMBER OF FIBERS UPON WHICH THIS CORRELATION IS BASED, AND FINALLY, FOR THE LARGER CORRELATIONS, THE PROBABILITY THAT A CORRELATION OF THIS MAGNITUDE WOULD OCCUR BY CHANCE IF THE CORRELATION WERE ACTUALLY ZERO.

LiCl	CaCl$_2$	NH$_4$Cl	KCl	HCl	QHCl	1.0 M Sucrose	
+0.94	−0.30	−0.11	−0.09	+0.39	+0.20	+0.17	
12	11	25	25	17	18	10	0.1 M NaCl
<0.01							
	−0.32	−0.30	−0.21	+0.31	+0.15	+0.46	
	11	14	14	12	13	11	0.1 M LiCl
		+0.77	+0.74	+0.45	−0.22	−0.25	
		12	12	12	11	10	0.3 M CaCl$_2$
		<0.01	<0.01				
			+0.88	+0.58	+0.11	−0.19	
			28	18	21	14	0.1 M NH$_4$Cl
			<0.01	<0.05			
				+0.59	−0.09	−0.18	
				18	21	14	0.3 M KCl
				0.01			
					+0.39	−0.12	
					17	11	0.03 M HCl
						+0.13	
						14	0.01 M QHCl

the training stimulus or another salt was noted.   Being shocked for drinking KCl resulted in a depression of drinking rates for both KCl and NH$_4$Cl, but not so much depression of NaCl drinking.   Similarly, being shocked for drinking NH$_4$Cl resulted in a depression of drinking rates for both NH$_4$Cl and KCl, but not so much for NaCl.   The depression of drinking from being shocked for NaCl generalized somewhat to both the other salts, but not selectively more to one than the other.

In the second behavioral test, two rats each learned to avoid drinking one of the three salt solutions used in the first behavioral test in a three-tube test (total of six rats). Shocks (3mA) were applied between the metal drinking tube and the floor whenever the rat drank for about 2 sec.   Each rat was first trained to avoid one of the three salts, and then tested for

degree of avoidance of all three.   In both tests, the following concentra-
tions were used in a random fashion to insure that the discriminations
were not developed on the basis of intensity differences : KCl and NH_4Cl—
0.01, 0.03, and 0.1 M ; NaCl—0.003, 0.01, 0.03 and 0.1 M. The results are
similar to those obtained in the first behavioral test.   Depression of drinking
generalized between KCl and NH_4Cl, but not so much between either of
these and NaCl.

To summarize the results of the behavioral tests, responses learned to
KCl generalized to NH_4Cl and vice versa.   Responses learned to either
KCl or NH_4Cl generalized much less to NaCl and vice versa.   It appears
that, for the rat, KCl and NH_4Cl taste more nearly alike than either do
to NaCl.   Therefore, in addition to concluding that there are many fiber
types in gustation, one may conclude that *the neural message for gustatory
quality is a pattern made up of the amount of neural activity across many
neural elements.*

These data support an across-fiber pattern theory for taste quality
sensitivity.   These patterns which signal the quality of the taste stimulus
are developed across a great number of fibers.   The various fibers involved
show considerable diversity in their sensitivity to taste stimulation ; this
diversity in sensitivity prevents easy fiber classification, but provides the
basis for the across-fiber patterns.

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TASTE FUNCTIONS IN FISH

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THIS paper deals with the electric activity of gustatory fibres of freshwater
fish and especially with the problem of specific receptors responding to
substances which may attract fish.

Hoagland (1933) was the first to record nerve discharges from gustatory
fibres in the facial nerve which innervate the barbels of the catfish. He
showed that chemicals initiate nerve impulses that are of smaller spike
height than those produced by mechanical stimulation. Recently we found
that the barbels are much less sensitive to chemical than to mechanical
stimulation (Konishi and Zotterman, 1961). In the roof of the carp's
mouth particularly dense taste buds are found. This palatal organ is
supplied by nerve fibres from the palatine nerve. Responses led from the
nerve evoked by the application of sapid substances were found to be very
strong. Besides chemoceptive and motor fibres the palatine nerves con-
tain fibres which respond very selectively to mechanical stimulation. The
integrated response to touch is, generally, much smaller than that to
chemical stimulation.

The palatine nerves in Swedish carp responded to all four conventional
classes of taste substances such as salt, sucrose, quinine and acid—especi-
ally strongly to acid (0.005 M/pH 3.8/acetic acid) and sucrose (0.5 M), and
weakly to quinine (0.01 M) (Fig. 1). Water did not stimulate the chemo-
ceptors of this fish, after a previous rinse with water. The low sensitivity
of fish to quinine is in close agreement with Trudel's behavioural results on
minnows (1929). Generally, quinine receptors adapted very quickly with
repeated application, whereas the others did not. In all the nerve pre-
parations tested, glycol and glycerol (which taste sweet to humans) elicited
a positive response, while saccharine gave a very feeble response if any. It
is of particular interest that human saliva had a strong stimulating effect
on the chemoceptors, and gave a much larger integrated response than the
0.5 M NaCl solution. Earthworm and milk also produced massive res-
ponses. The responses to sapid substances in Japanese carp were found to
be different from European, as shown in Fig. 2. Response to sucrose
(0.5 M) was always quite small, while quinine (0.01 M) gave a much larger

response than NaCl (0.5 M) and acetic acid (0.005 M). In European carp,
the stimulating effects of human saliva, earthworm-extract and milk do not

FIG. 1. Electrical responses from the entire palatine nerve of the Swedish
carp to the application of various solutions to the palatal organ and to touch.
In each tracing are recorded, from top to bottom : the signal showing the
moment of application, the integrated response, and the direct spike response.
Records N and O were recorded by high amplification. Time in seconds.
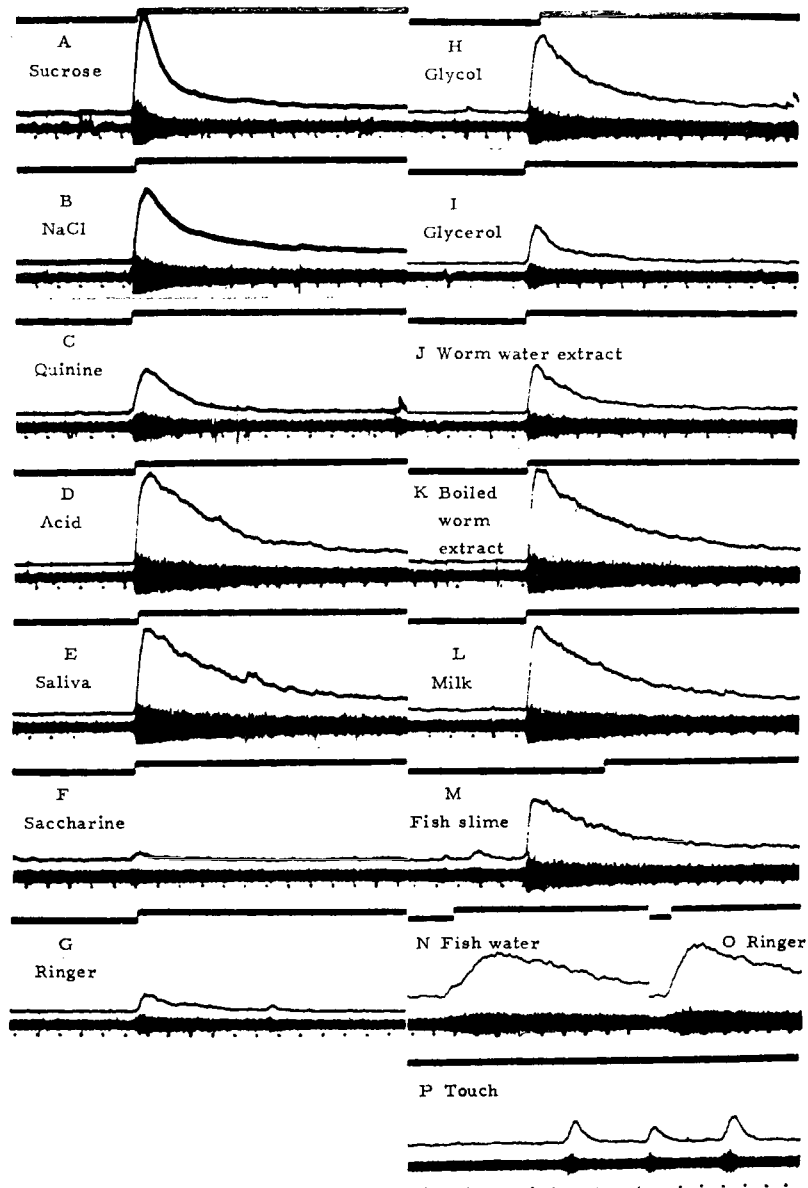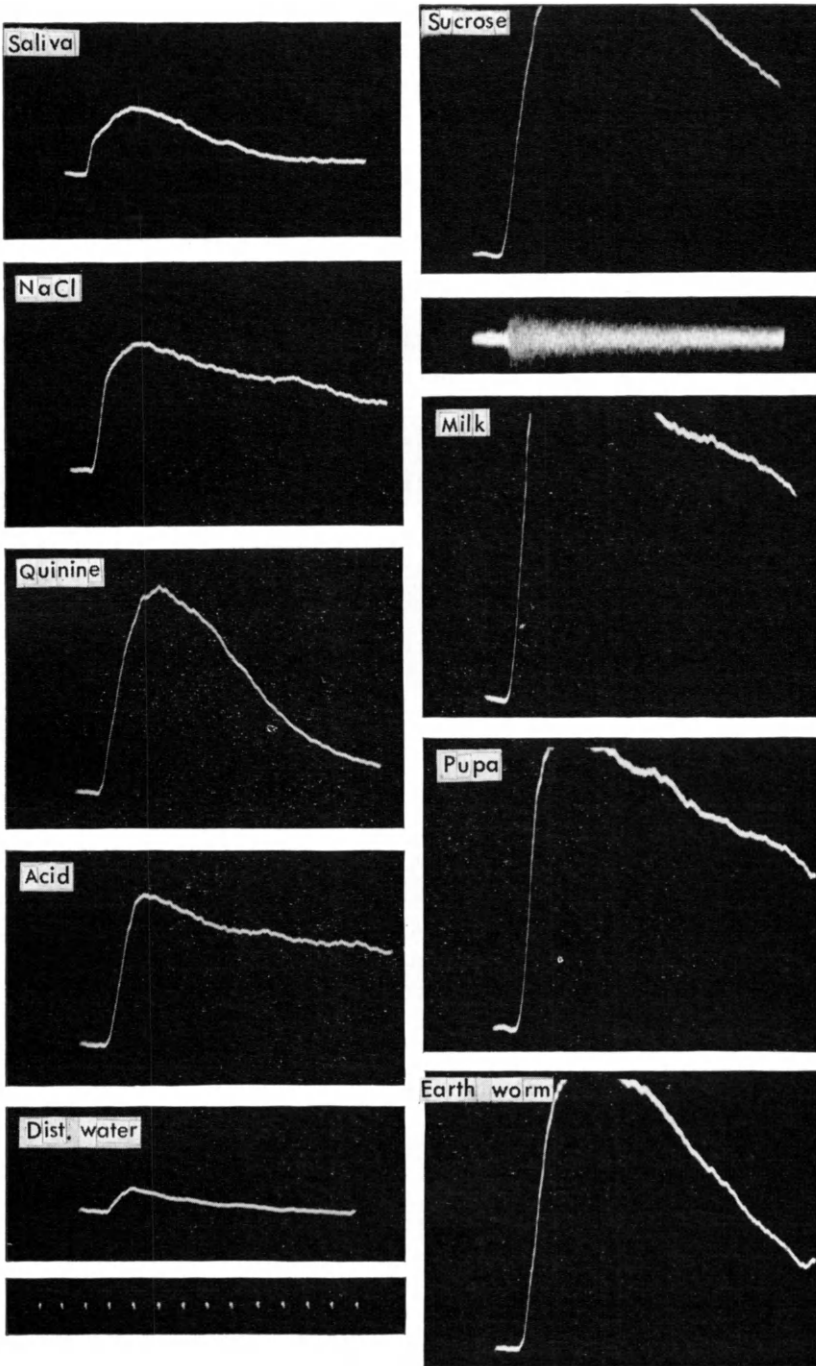
FIG. 2.   Integrated responses from the entire palatine nerve of Japanese carp
to the application of various solutions to the palatal organ.   Time in 0.5 sec.

exceed those of acid and sucrose, whereas Japanese carp showed far higher sensitivity to the saliva, earthworm-extract and milk as well as to extracts of silkworm pupa which is an important bait for carp in Japan.

The responses to sapid substances in the facial nerve from the barbel, and also the branchial nerve, innervating the chemoceptors on the gill rakers in this fish, were quite small. However, all taste substances which stimulated the palatal organ were able to produce positive responses in these nerves. On the other hand, tactile stimulation caused larger response than the response to chemicals.

A study of the records from single taste fibres in the palatine nerves revealed that the individual fibres could be divided into seven groups according to their response pattern. None of the taste fibres tested responded to tactile stimulation of the palatal organ. The number of touch fibres seems to be relatively small compared with the chemoceptive fibres. The characteristic of the gustatory fibres in this fish may be caused by fibres which respond to many taste substances in contrast to other animals such as the cat. The existence of many fibres displaying a broad gustatory spectrum draws attention to the possibility of a simple osmotic effect as the primary mechanism for producing taste sensation in the fish. This possibility, however, is ruled out because human saliva which is nearly isotonic to fish body fluid was intensively stimulating. We found specific taste fibres for salt and for acid (Fig. 5). It is especially noteworthy that among the 16 fibres of the salt fibre type so far studied, all except two were not stimulated by acid at the concentration used. This is the only taste fibre type which was insensitive to acid. Next to the fibre type displaying a broad gustatory spectrum the " sweet fibre " was commonly found (Fig. 4). Sweet tasting substances except for saccharine stimulated this fibre. It is particularly remarkable that human saliva selectively stimulated these " sweet fibres " as strongly as 0.5 M sucrose, and in some cases even more so. The salt, quinine and acid fibres were not stimulated by saliva. Besides the " sweet fibres " and the fibre displaying a broad gustatory spectrum, fibres which showed a vigorous response exclusively to saliva were rarely found (Fig. 6). The response pattern of individual palatine chemoceptive fibres are summarized in Table 1 with the exception of rare cases. The fact that the great majority of taste fibres running to the palatal organ of the carp display a rather broad gustatory spectrum raises again the question, previously brought up by Pfaffmann (1941) as well as by one of us (Zotterman, 1956), whether a taste fibre might possess in its endings different receptors responding to different classes of gustatory stimuli. As it is difficult to find any common denominator for the excitatory effect of such stimuli as sugar, salt, quinine and acid, it seems more likely that the broad gustatory sensitivity of these fibres is due to the existence of different chemosensitive sites in the villi of the gustatory cells or the fine endings of the individual

FIG. 3. Response of single palatine nerve fibre classified under the type I (b) to the application of various solutions to the palatal organ. Record F, responses of single touch fibre in the palatine nerve to the tactile stimulations. A signal shows the moment of application of the test solution. Time in seconds.

TABLE 1

Stimulus	NaCl (0.5 M)	Acetic acid (0.005 M)	Quinine (0.01 M)	Sucrose (0.5 M)	Saliva	Number of preparations
I a	+	+	−	+	+	31
b	+	+	−	+	−	6
II	+	+	+	+	+	21
III a	−	+	−	+	+	19
b	−	+	−	+	−	2
IV a	+	−	−	−	−	14
b	+	+	−	−	−	2
V	−	+	+	−	−	13
VI	−	(+)	−	−	+	3
VII	−	+	−	−	−	3

FIG. 4.   Response of single palatal nerve fibre classified under the type III to the application of various solutions to the palatal organ.   This is an example of grouped discharges.   Time in seconds.
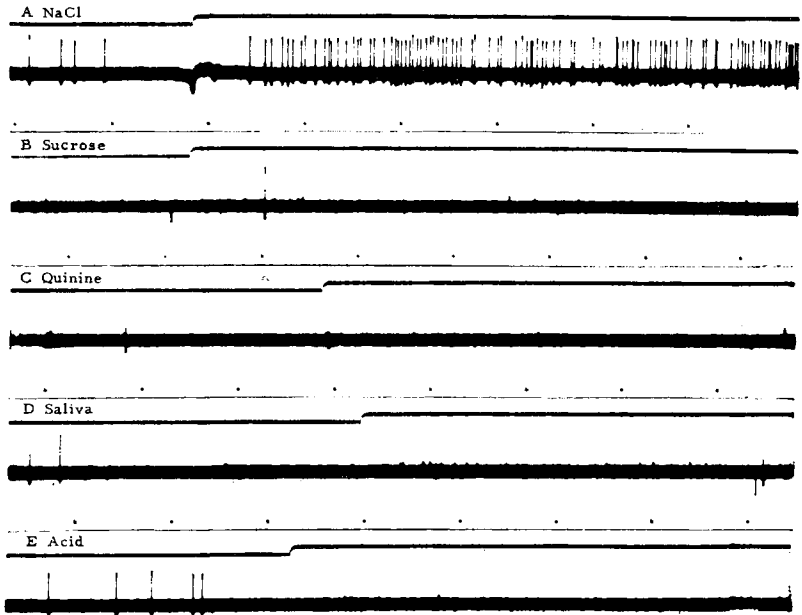
FIG. 5.   Response of single palatine nerve fibre classified under the type IV to the application of various solutions to the palatal organ.   Note the specific response to NaCl, and no response even to acid.   Time in seconds.

gustatory nerve fibres. Such an arrangement would thus be similar to Granit's system of dominators in the retina. Specific taste fibres corresponding to Granit's modulators we found only to salt, acid and to human saliva. In addition to these we have the fibre type which responds only to quinine and acid (type V) and type III fibres. From these findings it is possible to picture a mechanism enabling the fish to elaborate a fairly high level of gustatory discrimination.
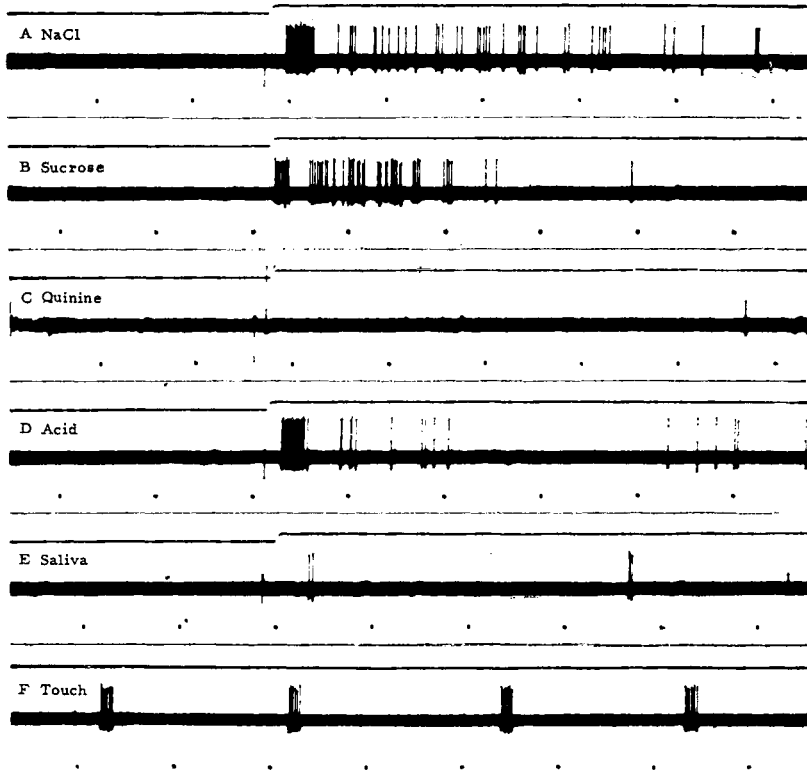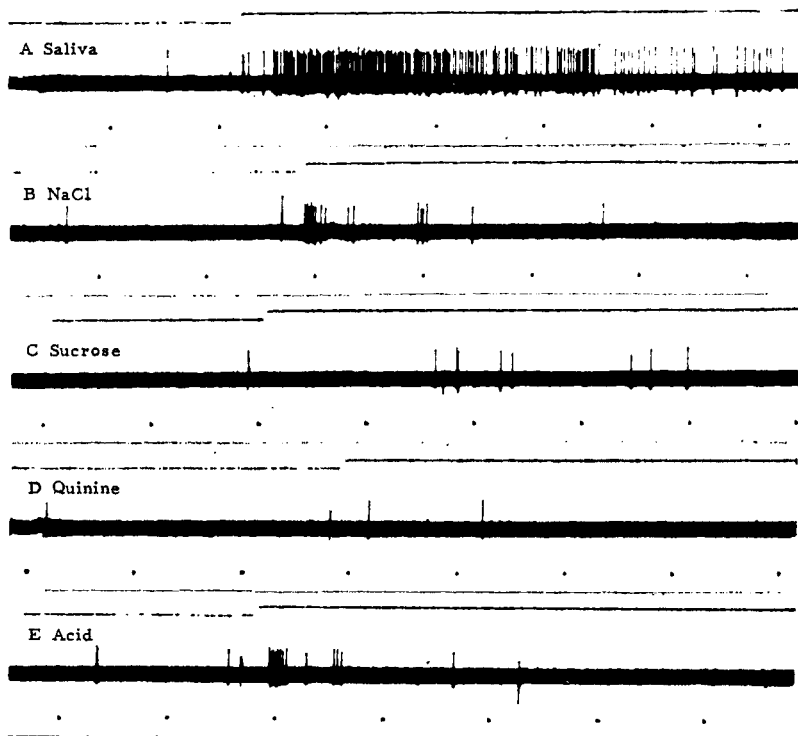

FIG. 6. Response of single palatine nerve fibre classified under the type VI to the application of various solutions to the palatal organ. Time in seconds.

The question naturally arises as to whether the fish has receptors which respond to water or not. Chemoceptors of the fresh water fish are naturally exposed to water. All of the foregoing experiments, therefore, have been undertaken with fish which were adapted to water. Under this condition no fibre responding to water was observed. However, water sometimes produced a response when applied shortly after a previous application of salt (or even Ringer's solution), sucrose, acid, and especially strongly after saliva. This response to water, however, is rather different from the water response reported in other animals with respect to the following fact : The response to water in the fish was only seen after the previous application of

salt or non-electrolytes. Figure 7 illustrates the integrated responses to varying concentrations of NaCl and sodium acetate of fishes adapted to water or to isotonic saline solution for more than one week. No response to distilled water was observed in the fish adapted to isotonic saline. The
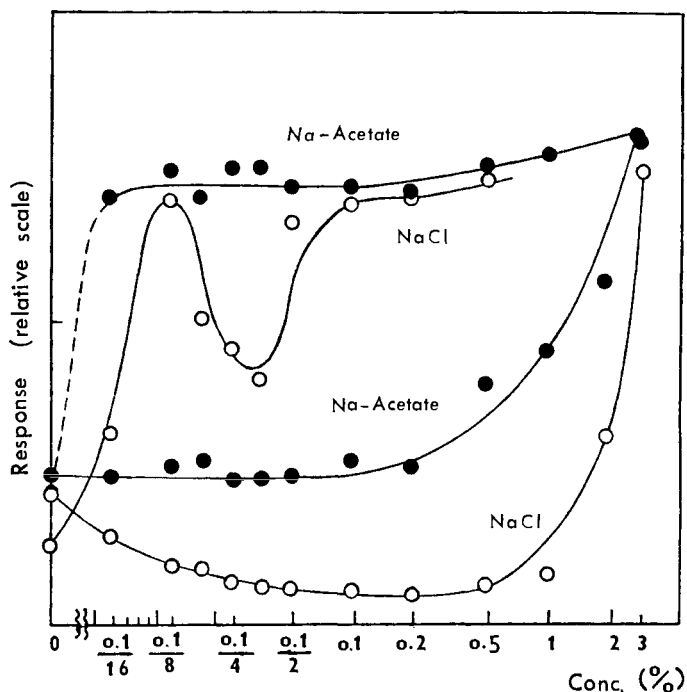
FIG. 7. Response of the entire palatine nerve of the carp to varying concentrations of NaCl (open circles) and sodium acetate (solid circles). Upper two curves, responses from the carp adapted to water ; Lower two curves, responses from the carp adapted to isotonic saline more than one week.

mechanism involved in the positive response caused by a water stimulus after a previous application of salt is not yet clear. From our experiments it seems unlikely that it is the outflow of ions through the receptor membrane which affects the receptor excitability in the fish. The excitation produced by water must be mediated by a rather different mechanism than that involved in the so-called water response in other animals. The fact that a previous application of non-electrolytes, which hardly can diffuse into the receptor cell, also elicited large responses to water, even much larger than that after salt, seems to support such a view.

As is seen from Fig. 7, the fibres in the fish adapted to isotonic saline show responses to dilute NaCl solutions that are rather smaller than the responses to distilled water, indicating a depression of the effect of NaCl, while sodium acetate almost has no depressing action. In this respect the

responses to various concentrations of salts resemble those of the " water fibre " in the cat described by one of us (Zotterman, 1955), and thus it seems likely that the comparatively weak response to water observed in the fishes adapted to isotonic saline might be induced by the mechanism similar to so-called water response found in cat, etc. It is particularly of interest to note that in the fish adapted to water, the depressing action of salt hardly occurs at the low concentration as shown in Fig. 7.

It was previously stated that human saliva has a strong stimulating effect on the chemoceptors in carp, especially in the Japanese carp. Single fibre experiments revealed fibres responding only to saliva. This finding suggests the possibility that the saliva would selectively stimulate receptors which are sensitive specifically to an unknown substance in the saliva. Experiments were undertaken in the hope of finding the gustatory active substance in the saliva. The fact that human saliva is a powerful stimulant of the fish palatal organ gives us of course a new aspect on the angler's habit of spitting on the bait.

The fact that saliva did not stimulate the " salt fibres " excludes the possibility that the electrolytes dissolved in the saliva may serve as the effective stimulating agent. In fact, salivary electrolytes such as KCl, various phosphates, KCNS, KI and KBr could not give effective responses at concentrations similar to those found in saliva (Fig. 8). Salivary enzymes, such as ptyalin, lipase and phosphatase, could not be regarded as the effective stimulant, as we found that saliva had the same stimulating effect after heating (Fig. 8). Mucin in the saliva does not seem to be the active substance, because mucin-free saliva had a strong effect, as will be mentioned later. The cattle's submaxillary mucin* and also the mixed solution of mucin and electrolytes produced only small responses (Fig. 8). The composition of the submaxillary mucin of cattle is as follows :

Sialic acid : ca. 30% Hexosamine : 13% Hexose : 3%.

The saliva contains many kinds of amino acids and vitamins at extremely low concentration (Hinsberg and Schmid, 1953, and Kawamura, 1957). A series of different amino acids and vitamins were tested, but all were found to be inactive in low concentrations (Fig. 8). Neither alone nor mixed could these amino acids or vitamins stimulate as strongly as natural saliva. The substances tested were as follows :

Amino acid : 1-tryptophan, 1-arginine, 1-valine, 1-glutamic acid, 1-phenyl alanine, 1-threonine, 1-lysine, 1-glycine, 1-tyrosine, 1-isoleucine, 1-proline, 1-leucine, 1-serine, 1-cystine, 1-histidine, 1-methionine, taurine.

Vitamins : aneurin, pyridoxine, biotine, lactoflavin, pantothenic acid, ascorbic acid, nicotinic acid, folic acid, vitamin K.

It is interesting to note that 1-serine, which is said to be a powerful olfactory stimulant for the salmon (Idler et al., 1956), although it is

* For samples of pure mucin we are indebted to Prof. G. Blix, Uppsala.

repellent, could not be distinguished from other inactive amino acids in its gustatory stimulating effect. Urea, creatinine, NH_3, lactic acid, citric acid, cholesterine, bradykinin, kallikrein and parotine, salivary gland hormone, all displayed a weak effect in low concentrations (Fig. 8).

FIG. 8. Electrical responses from the carp's palatine nerve to the application of the solutions of different substances which are contained in the human saliva to the palatal organ. A, human saliva ; B, 0.2% mixed solution of salts (0.0836 g % KCl, 0.0468 g % K_2HPO_4, 0.0372 g % Na_2HPO_4, 0.0203 g % $CaHPO_4$ and 0.00074 g % $MgHPO_4$) ; C, 0.2% cattle submaxillary mucin in 0.2% salt sol. ; D, 0.2% submaxillary mucin and 0.004% KCNS in 0.2% salt sol. ; E, human saliva ; F, boiled and filtrated saliva ; G, 3 mg % glycine ; H, 3 mg % glutamic acid ; I. 3 mg % serine ; J, mixed solution of vitamins ; K, 0.1 M glucose ; L, 10 mg % lactic acid ; M, 10 mg % creatinine ; N, cholesterine ; O, 30 mg % urea ; P, 20 B.U. % kallikrein ; Q, 5 mg % parotine. Time in seconds.

In order to find the active substance, experiments were performed from another direction. Saliva was fractionated by organic solvents. To the saliva was added an excess of 99.5 per cent ethanol, and the precipitates of

protein, mucin and other ethanol insoluble products were separated from the ethanol soluble fraction. The ethanol soluble and insoluble fractions were then dried by evaporation and distilled water was added to the original volume of the saliva. The aqueous solution of the ethanol soluble fraction was still as active a gustatory stimulant as the natural saliva, while the ethanol insoluble fraction had a very weak activity (Fig. 9). Renewed ethanol treatment yielded a precipitate which shows a weak Molisch's test, and the ninhydrin test was always negative. The aqueous solution of these precipitates produced only a weak response. Similarly, methanol soluble fraction stimulated very strongly, although somewhat weaker than the ethanol soluble fraction. Similar treatment with acetone, however, showed that the powerful stimulating agent in saliva is insoluble in acetone. The active substance seems to be hardly destroyed by these treatments and it is also resistant to heating. Repeated treatments with ethanol followed by acetone demonstrated that the gustatory active substance always occurs in the ethanol soluble and acetone insoluble fraction. While amino acids, primary amines and proteins were not detected in either the gustatory active fraction or the inactive fraction, traces of carbohydrates were demonstrated in both fractions by Molisch's test. We first guessed that the gustatory active substance in saliva might be a substance chemically related to the phospholipid group from the following facts : (1) organic phosphorus was detected in the acetone insoluble fraction, and (2) the addition of $CdCl_2$ yielded a small amount of precipitate from the alcoholic solution of the acetone insoluble fraction but not from the acetone soluble fraction. The treatment with ether following ethanol, however, revealed that the active substance appears in the ether insoluble fraction (Fig. 9). This clearly indicates that the active substance is at least not related to a glycerophospholipid. Since blood group substances in saliva are alcohol-insoluble carbohydrates (Kabat, 1956), it seems unlikely that they are involved in the gustatory excitation.

The observation that silkworm pupa, earthworm and milk had strong stimulating effects similar to the saliva, indicates that there may be gustatory active substances in these sapid materials. In order to investigate the problem, similar fractionation with organic solvents was performed (Fig. 9). The results clearly showed that all active extracts of these sapid substances occur in the ethanol-soluble and acetone-insoluble fractions as was the case with saliva. The question whether the active agent in these sapid substances would actually stimulate the receptors which are stimulated by saliva was then investigated. For that purpose the technique of selective adaptation was introduced. Application of saliva to the receptive field of the palatal organ which was adapted to milk or the pupa fluid produced only a small response, while the saliva response was not markedly changed by previous adaptation to the solutions of salt, quinine and acid (Fig. 11—I). A similar
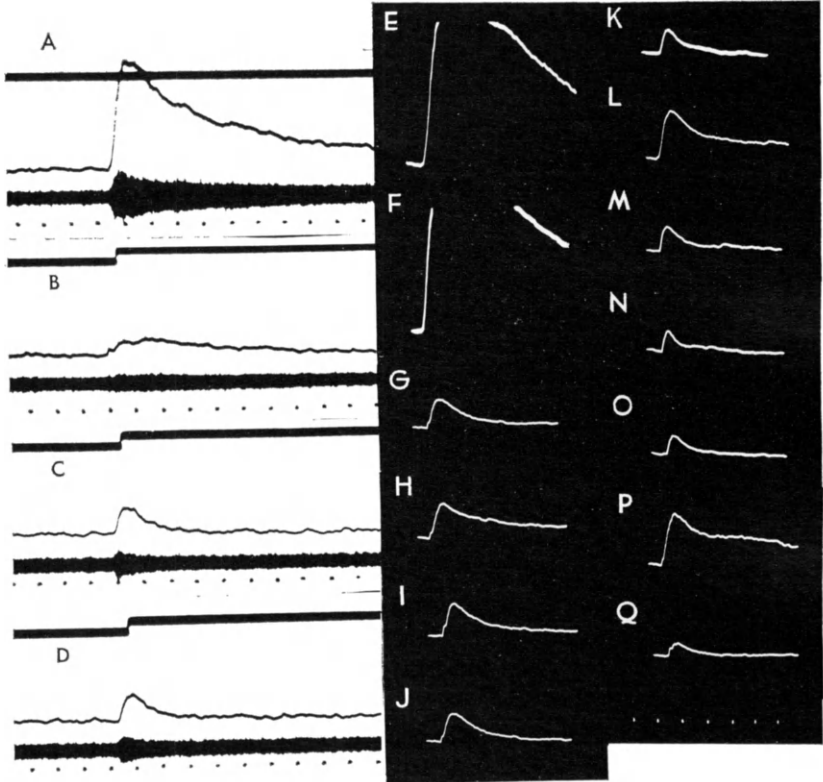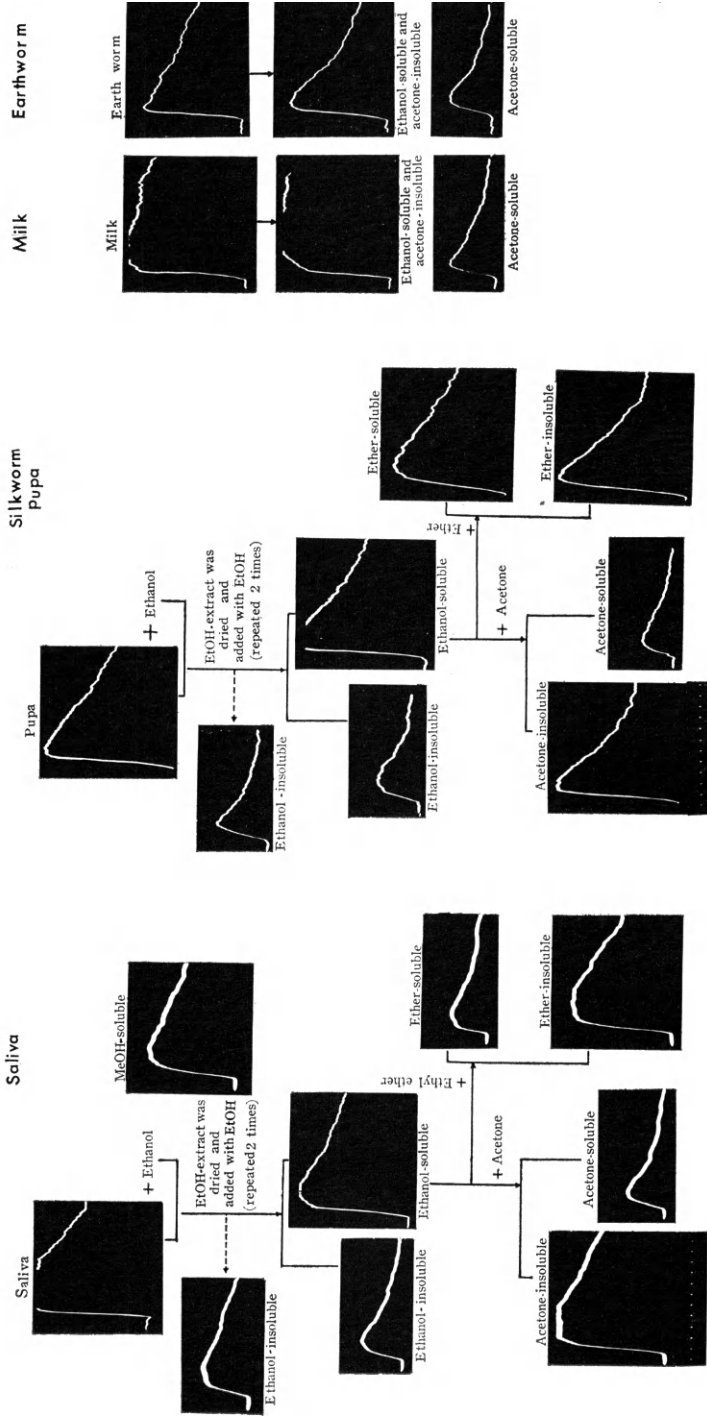
FIG. 9. Electrical responses from the carp's palatine nerve to the application of the solutions of different components of sapid substances fractionated by organic solvents. All fractions were dried and made up in distilled water. Time in 0.5 sec.

selective adaptation was also found in the response to the pupa fluid follow-
ing previous application of the saliva (Fig. 11—II). These findings strongly
suggest that the receptors responding to saliva are commonly stimulated
by the sapid substances mentioned above and, in addition, the main
gustatory active component in all these substances probably belongs to the
same or a similar group. However, the fractionation of the residue of the
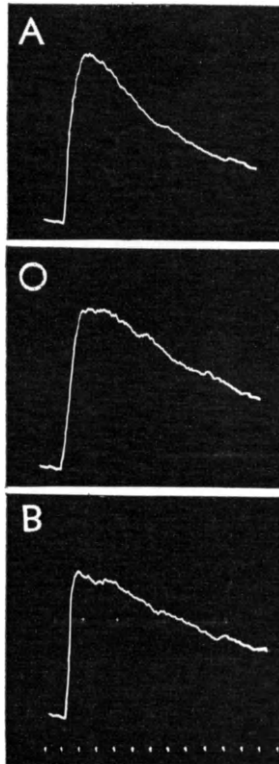
FIG. 10. Electrical response from the carp's palatine nerve to the application
of the human saliva which contains specific blood group substance. A, saliva
with blood group substance of type A ; O, saliva with that of type O ; B, saliva
with that of type B. Time in 0.5 sec.

ethanol soluble fraction of the pupa by ether revealed that the gustatory
active substance occurs in both the ether soluble and insoluble fractions,
suggesting the presence of at least two main active components in the pupa,
and that one of them might belong to a group similar or related to that in
saliva (Fig. 9). Both fractions had a potency similar to the original pupa
extract. Molisch' reaction and Bial's orcin reaction were strongly positive
in the ether insoluble fraction, and very weak positive in the soluble
fraction.

The infrared analyses of both the above mentioned ether soluble and acetone insoluble fractions of pupa demonstrated absorption bands which are characteristic for glycerophospholipid. The spectrum possesses strong bands of ester C=O stretching, P=O linkage and other bands which may
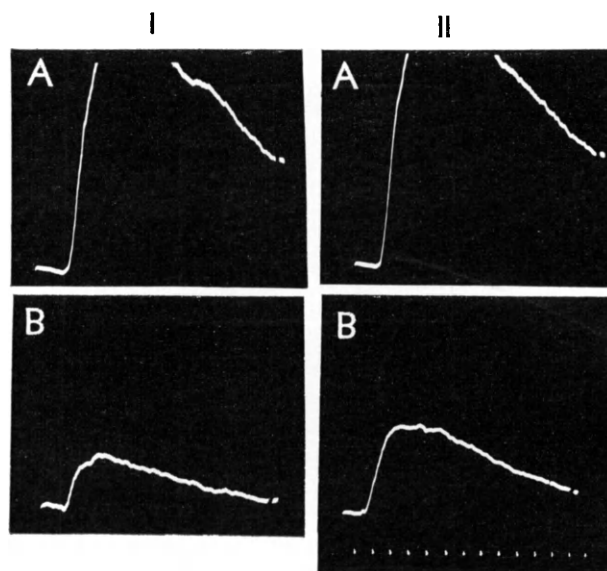
FIG. 11. Showing the selective adaptation. I–A, response to human saliva ; I—B, response to the application of the saliva following previous adaptation to milk ; II—A, response to silkworm pupa fluid, II—B, response to the application of the pupa fluid following previous adaptation to human saliva. Time in 0.5 sec.
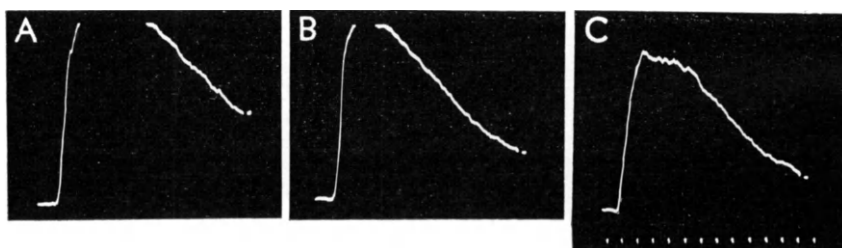
FIG. 12. Electrical response from the carp's palatine nerve to the application of lecithine to the palatal organ. A, pupa fluid ; B, 0.5 per cent lecithine from yolk ; C, 0.5 per cent lecithine from soya bean. Time in 0.5 sec.

serve as reference peaks for the identification of glycerophospholipids (Freeman *et al.*, 1953 ; Marinetti and Stotz, 1954), but lacks the absorption bands which are assigned to monosubstituted amide groups. On the other hand, the spectrum from the ether insoluble fraction showed strong absorption bands of the monosubstituted amide group and very strong OH and

NH bands in addition to phosphate linkage. The possible assignments of the bands observed in the spectra of these gustatory active fractions are shown in Table 2. From these analyses it seems most probable that the gustatory active substance from the pupa which is soluble both in ethanol

TABLE 2.   WAVELENGTHS OF PEAK VALUES FROM INFRARED ABSORPTION SPECTRA FOR GUSTATORY ACTIVE FRACTIONS OF HUMAN SALIVA AND SILKWORM PUPA EXTRACT.

Possible assignments	I	II
OH, NH stretching	3.1μ	2.95μ
CH stretching	3.45	3.4
Amide C=O stretching	6.1	6.05
Amide NH deformation	6.3–6.6	6.2–6.4
CH_2, CH_3 bending	7.15	7.0
Acetal C—O—C linkage (or P=O)	8.06	8.05
P=O	9.3	?
Trans double bond (and P=O)	10.4	10.3
Long chain hydrocarbon	14.0	13.4

Possible assignments	III
OH (NH) stretching	3.08μ
CH stretching	3.5
Ester C=O stretching	5.8
CH_2, CH_3 bending	6.85
Ester C—O—C linkage and P=O	8.1
P=O	9.2
P=O	10.3
Long chain hydrocarbon	13.8

I : Ethanol soluble and ether insoluble fraction of pupa.
II : Ethanol soluble and ether insoluble fraction of saliva.
III : Ethanol soluble and ether soluble fraction of pupa.

and in ether may be related to glycerophospholipid. Whereas the other active substances which are ethanol soluble, but insoluble in ether, may be sphingolipid-like, probably a mixture of sphingomyelin and glycolipid. The spectrum from the ethanol soluble and acetone insoluble fraction of pupa which possessed both ester C=O and amide bands might indicate that these fractions contain both glycerophospholipid and sphingolipid. Although the gustatory active substance in the human saliva could not be identified and further extensive analyses are necessary, it seems likely from the infrared analysis of the ethanol soluble and ether insoluble fraction of saliva that the activity might be exerted by certain lipid-like substances which contain an amide group and probably also carbohydrates. According

to Funakoshi (1962), the active substance in saliva may be a macromole-
cular one which is non-dialzyable through Cellophane membranes.

It is thus most probable that there may be more than two kinds of gusta-
tory active lipid-like substances in the sapid materials which strongly
stimulate fish chemoceptors. The view that one of the active substances
in the pupa belong to the glycerophospholipid group is fortified by the
facts that (1) 0.5 per cent lecithine, both from yolk and soya bean, was a
strong stimulus (Fig. 12), and (2) the response to lecithine was remarkably
affected by the previous adaptation to the pupa fluid. Unfortunately, we
have not yet tested pure preparations of sphingolipid or the related lipid
from other sources, and it is thus difficult at present to make a final identi-
fication of the gustatory active components in the ether insoluble fraction
of the pupa as well as those in saliva. At any rate, it looks as if lipid-like
substances, except for fatty oils, are capable of stimulating strongly the fish
chemoceptors. It is of interest to note Hiyama's observation (1947) that
rice-chul, common freshwater fish in Japan, preferred the hook smeared
with the fat of pupa of a certain moth to a hook without the fat. The
results presented here in addition to the finding of specific fibres responding
to the saliva suggest the existence of highly differentiated specific receptors
that respond to specific substances, though they may not exist in other
animals. Furthermore the results give us the impression that the majority
of the substances which attract fish may mainly stimulate these receptors.
We only found a few specific fibres responding to saliva in the Swedish carp,
but many more such fibres may be detectable, especially in the Japanese
carp, because Japanese carp have higher sensitivity to saliva than Swedish
carp, despite their less sensitivity to sugar.

The effects of lytic agents on the chemoceptor activity in the carp were
investigated. Digitonin had a strong influence on the chemoceptors, which
were impaired almost immediately even at as low a digitonin concentration
as 0.005 per cent. The action of sodium cholate was rather mild. Never-
theless it finally made the receptors almost inexcitable. It was found that
the sweet and saliva receptors were paralysed most quickly by the treatment
of 0.3 per cent sodium cholate ; the sweet receptors were affected almost
immediately while the salt and acid receptors were more resistant, all in
accordance with the action of gymnemic acid (Fig. 13). The quinine
receptors formed an intermediate group. The fact that digitonin, a typical
lytic agent, which probably acts exclusively on the lipid part of the cell
membrane (Danielli, 1951), could very quickly extinguish the chemoceptor
activity even at a very low concentration suggests that the chemoceptor
membrane in the fish may have a lipid layer in its molecular structure.
This layer may play an important role in gustatory excitation comparable
to excitation in nerve axon (Tobias, 1958) and in visual cells (Sjöstrand,
1953). If that is the case, our study of the gustatory active components in

several powerful sapid substances are interesting in connection with the following two points : (1) resemblance in molecular structures between the gustatory active lipid-like substances and the structural complex of the receptor membrane (2) saliva stimulates sweet fibres and the specific receptors responding to saliva, both of which were easily impaired by the
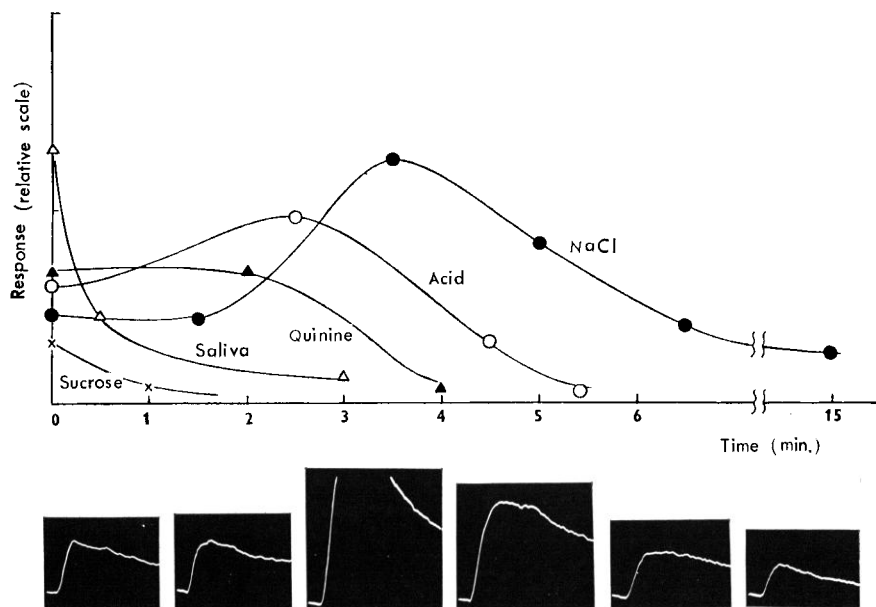
FIG. 13. Effect of sodium cholate (0.3 per cent) on the responses of the taste nerve endings of the carp to the application of various sapid solutions. Abscissa: Time after administration of sodium cholate. Series of lower records showing gradual shift in the response to the application of 0.5 M NaCl. Records from left to right are taken before application of sodium cholate, 1′ 30″, 3′ 30″, 5′, 6′ 30″ and 15′ thereafter.

treatment of sodium cholate which probably acts mainly on the lipid layer of the membrane. It is especially noteworthy as is shown in Fig. 13 that there is a temporal enhancement of the responses to acid and salt before the endings are paralysed. This was not observed in the response to sugar and saliva. Strong resistance of the salt receptors to lytic agents was also observed in the treatment by silver nitrate. Although we have no direct evidence so far regarding the mechanism involved in this temporal enhancement, it seems possible that the lytic agents may loosen the structural complex of the receptor membrane resulting in a higher degree of excitability.

SUMMARY

1. Chemoceptive fibres of the palatine nerves innervating the palatal organ of carp were found to respond to a great variety of sapid solutions.

The application of water to the palatal organ had no stimulating effect, after a previous rinse with water. Swedish carp displayed a high gustatory sensitivity to sugar and acid, and low sensitivity to quinine, while Japanese carp had low sensitivity to sugar, but showed a high sensitivity to bitter substances. The nerve preparations also responded very strongly to human saliva. Earthworm, milk and silkworm pupa extract very strongly stimulated the chemoceptors of the fish.

2. A study of single fibre responses revealed that the fibres could be divided into seven groups according to their response pattern. Besides the fibres displaying a broad gustatory spectrum, saliva always stimulated fibres which responded positively to sugar. Fibres which responded to NaCl, acid, or quinine, did not respond to saliva ; those that responded to sugar did respond to saliva.

3. From experiments on selective adaptation, it was suggested that the receptors responding to saliva are commonly stimulated by the sapid substances which may attract fish, such as silkworm pupa extract. The existence of specific receptors highly differentiated in order to respond to specific components of powerful sapid substances such as the saliva is discussed.

4. Experiments were undertaken in order to find the gustatory active component in human saliva. Various substances which are already known to be present in the saliva could be eliminated. The final gustatory active component in the saliva could not be identified, but it is an alcohol soluble and ether and acetone insoluble substance which may contain mono-substituted amide groups, carbohydrates and probably also long chain hydrocarbons in its molecular structure. The active substance is not destroyed by boiling.

5. Fractionations with organic solvents revealed that silkworm pupa extract has at least two main gustatory active components. From infrared analyses, one of them might be a substance related to glycerophospholipid, and another probably a substance related to sphingolipid.

6. The effect of lytic agents on the chemoceptor activity in the fish were investigated. It was found that receptors responding to sugar and saliva are paralysed most quickly by treatment with sodium cholate, and that salt and acid receptors are more resistant to this lytic agent. Prior to the paralysing of the receptors, temporal enhancements of the sensitivity to salt and acid were observed.

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COMPARATIVE ANATOMICAL AND PHYSIOLOGICAL STUDIES OF GUSTATORY MECHANISMS*

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DURING the course of previous investigations variations in the route and structure of the chorda tympani nerve were observed within and among species. A study was initiated which was directed toward describing the gross and microscopic structure of the chorda tympani nerve and the peripheral distribution of the lingual branch of the mandibular nerve in selected domestic animals. A detailed report of the results of this study will be published elsewhere (Engebretsen and Kitchell, unpublished). Figures 1–4 illustrate some of the results of the gross anatomical studies.

In studies of the neural responses in the chorda tympani following the application of solutions to the tongue, the chorda tympani nerve is usually exposed in the infratemporal fossa by resection of the mandible caudal to the last molar tooth and disarticulation at the temporomandibular articulation. Removal of the articular disk facilitates observation of the chorda tympani nerve. Certain comparative anatomical features are of particular importance. The chorda tympani nerve in the pig (Fig. 2) is closely associated with the mandibular branch of the trigeminal nerve from the point the mandibular nerve leaves the cranial cavity through the oval notch of the foramen lacerum until the chorda tympani nerve intermingles with the lingual branch of the mandibular. In order to locate the chorda tympani nerve in the pig the auriculo-temporal and mandibulo-alveolar nerves must be severed and reflected rostrally. The nerve can be seen by dissecting the mandibulo-alveolar nerve centrally. The chorda tympani nerve appears as one or two fascicles crossing the medial surface of the mandibulo-alveolar nerve at right angles to other fascicles in the mandibulo-alveolar nerve. The chorda tympani in the pig has only a very short portion whereby it is completely separate from the mandibular nerve before the chorda tympani enters the petrotympanic fissure.

The chorda tympani nerve in cattle has quite different relationships. The course of the nerve is often aberrant. It may join the lingual distal to
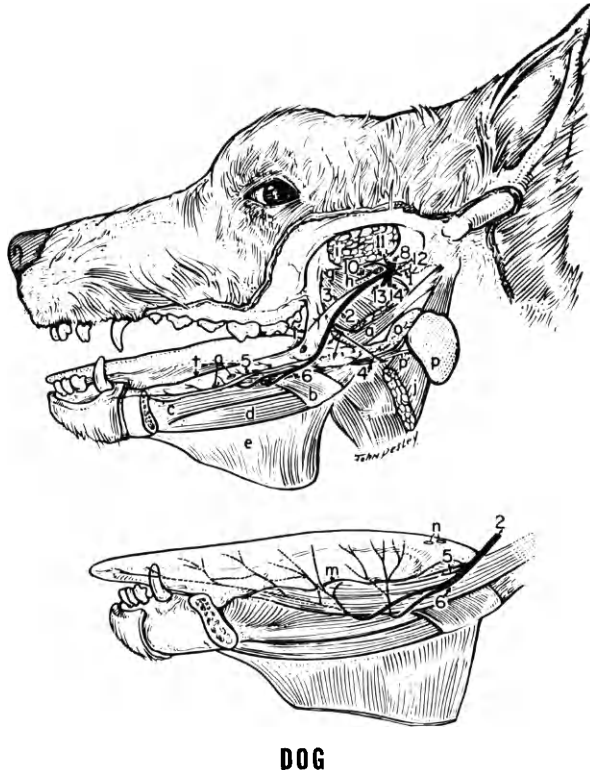
DOG

FIG. 1.   Gross dissection left mandibular and left lingual nerves in the dog. Most of the mandible has been removed to permit illustration of the branches of the mandibular nerve (N. mandibularis PNA), (Engebretsen and Kitchell, unpublished).

Legend Figs. 1–4

1, Chorda tympani n.   2, Lingual n.   3, Branch to isthmus faucium.   4, Parasympathetic innervation of the submandibular salivary gland.   5, Sublingual n. 6, Lingual n. before dividing into lingual branches.   7, Branch to sublingual salivary gland.   8, Mandibular n.   9, Pterygoid nn.   10, Buccal n.   11, Common masseter and deep temporal n.   12, Auriculotemporal n.   13, Mandibulo-alveolar n.   14, Mylohyoid n.   a, Styloglossus m.   b, Hyoglossus m.   c, Genio-glossus m.   d, Geniohyoideus m.   e, Mylohyoideus m.   f, Stylohyoideus m. g, Medial pterygoid m.   h, Lateral pterygoid m.   i, Temporal m.   j, Diagastric m. k, Masseter m.   m, Cut edge mucous membrane of the tongue.   n, Vallate papillae.   o, Sublingual salivary gland.   p, Submandibular salivary gland.   p′ Submandibular salivary duct.   q, Diverticulum auditory tube.   t, and t′   Cut edge of mucuous membrane along the mandible.

the rostral border of the medial pterygoid muscle.   In these instances it may lie medial to the medial pterygoid muscle and may be four or more

centimeters in length. The usual course of the chorda tympani nerve in cattle is illustrated in Fig. 3.

The peripheral distribution of the lingual branch of the mandibular nerve is illustrated in Figs. 1–4. Three branches of this nerve, the branch (or branches) to the isthmus faucium (no. 3, Figs. 1–4), the sublingual nerve
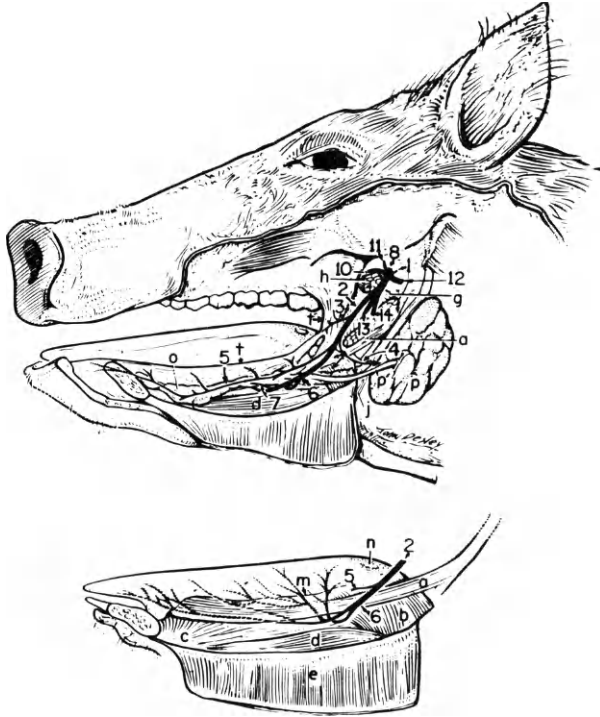
PIG

FIG. 2. Gross dissection left mandibular and left lingual nerves in the pig. For legend see Fig. 1.

(no. 5, Figs. 1–4) and the branch which supplies the parasympathetic innervation to the submandibular salivary gland (no. 4, Figs. 1–4) go primarily to structures other than the tongue. Centrally, fibers from these branches leave the lingual and run in the chorda tympani nerve.

The branch (or branches) to the isthmus faucium divides from the lingual nerve at the rostral border of the medial pterygoid muscle. Some branches go to oral gingiva on the medial surface of the mandible adjacent to the last cheek tooth and others go to the glossopalatine fold and the soft palate.

The sublingual nerve innervates the oral gingiva on the medial surface of the mandible adjacent to the molar and pre-molar teeth. Rostral

branches of this nerve can be traced to the oral mucosa of the medial sur-
face of the mandible caudal to the incisor and canine teeth. It also sup-
plies the sublingual salivary gland and the oral mucosa ventral to the
tongue. In horses and cattle anastomoses between the sublingual and
lingual branches have been observed (Engebretsen and Kitchell, un-
published).

COW
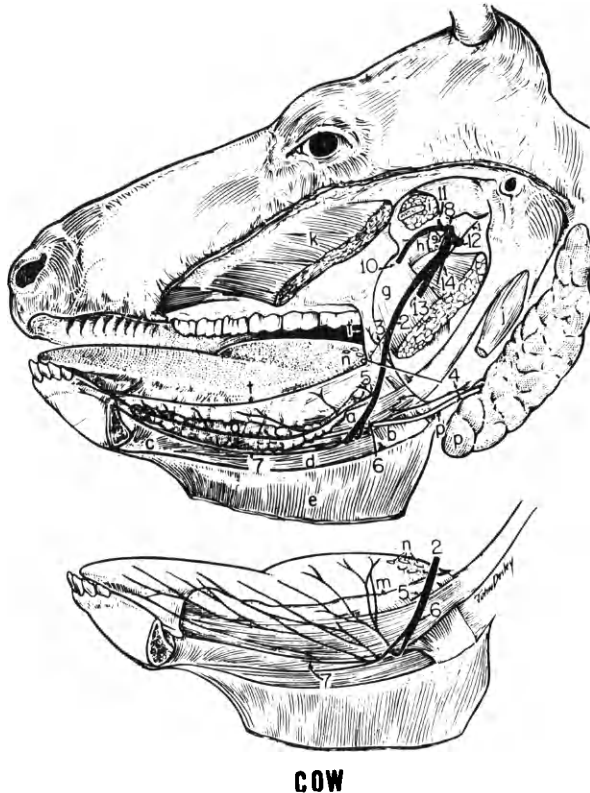
FIG. 3.   Gross dissection left mandibular and left lingual nerves in the cow.
For legend see Fig. 1.

The microscopic anatomy of the chorda tympani nerves from various
domestic animals has also been of interest to us. Immediately after the
exsanguination of an anesthetized animal the chorda tympani nerve was
dissected free from the surrounding tissue. The middle one-third of the
nerve was stretched gently across a rectangular orifice in a small piece of
cardboard. The ends of the nerve were made to adhere to the card-
board by gentle pressure. This procedure resulted in a mild degree of
longitudinal tension on the nerve during fixation in Flemming's solution
(1 per cent chronic acid 15 ml, 2 per cent osmic acid 4 ml and glacial acetic
acid 1 drop) for 24 hr. The nerves were dehydrated in dioxane, embedded

in paraffin and sectioned transversely at 6μ.   The sections were stained using the Wolters modification of the Weigert-Pal technique (Romeis, 1937).
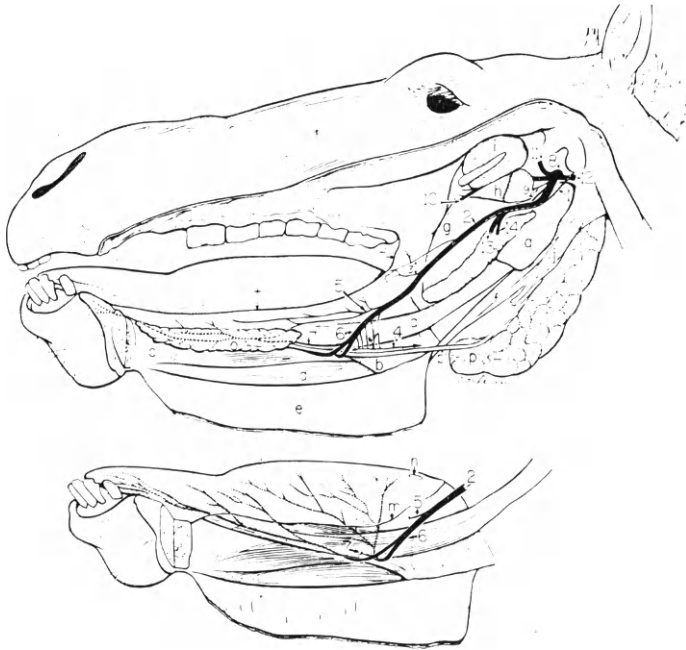
HORSE

FIG. 4.   Gross dissection left mandibular and left lingual nerves in the horse. For legend see Fig. 1.

The number and diameter of the myelinated fibers in each nerve were determined by measuring and counting the fibers as seen in a photomicrograph enlarged 750 times.   It was necessary to make composite photographs of the larger nerves (Figs. 6 and 7).   The equipment used in determining the diameter of each fiber and counting the fibers is illustrated in Fig. 5.   The gauge was prepared by etching a series of circles on clear Plexiglass.   The smallest circle was a 750 times enlargement of a circle having a 2μ diameter.   Additional circles were made, each of which compared to circles enlarged 2μ in diameter.   The largest circle corresponded to the enlargement of a circle 22μ in diameter.   The diameter of each myelinated fiber was measured and the fiber counted in the following manner.   The smallest circle of the gauge was placed over the photographic enlargement of the fibers.   If the outer edge of the fiber was equal to or smaller than the etched circle, the fiber was counted and marked by puncturing the photograph using a needle inserted through a hole in the

center of each etched circle of the Plexiglass gauge. The needle actuated a
mechanical counter. After all the fibers in the photograph of the same size
or smaller than the smallest circle were marked and counted, the number
was recorded and the next circle on the gauge was used in a similar manner.
In fasciculated nerves, the data was recorded for each fasciculus.
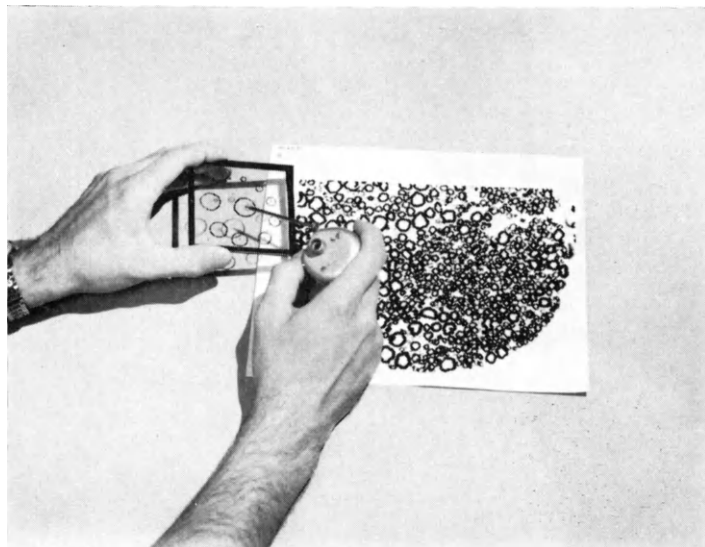
FIG. 5. Photograph illustrating method of counting fibers and determining
their diameter. The circles of the gauge are outlined in black for illustrating
only. The mechanical counter is held in the right hand. The pointed tip of
the counter is inserted through the small hole in the center of each circle and
into the photographed enlargement of a fiber of corresponding diameter; thus
sizing, marking and counting is accomplished.

As a control, direct measurements of the diameters of selected and
identifiable fibers were made from the microscopic sections using a cali-
brated ocular micrometer and the high power of a microscope. These
measurements were made by a skilled technician without knowledge of the
results obtained by the photographic procedure described above. These
control measurements revealed no significant differences between the two
methods of measurement.

The chorda tympani nerves of dogs and cats were not fasciculated
whereas those of the other domestic animals were fasciculated (Figs. 6, 7,
and 8). The number of fascicules present in different cross-sections of the
nerve varied (Fig. 8). Anastomoses between fasciculi occurred at irregular
intervals. The appearance of a perineural trabeculus indicated the for-
mation of a new fasciculus or an anastomoses between two fasciculi. The
chorda tympani nerves in some sections in the pig were observed to be

non-fasciculated with a number of perineural trabeculae (Fig. 6) but in adjacent sections were found to have two or three fasciculi.   In the chorda tympani nerves of the dog and cat, large fibers remained in the same quad-
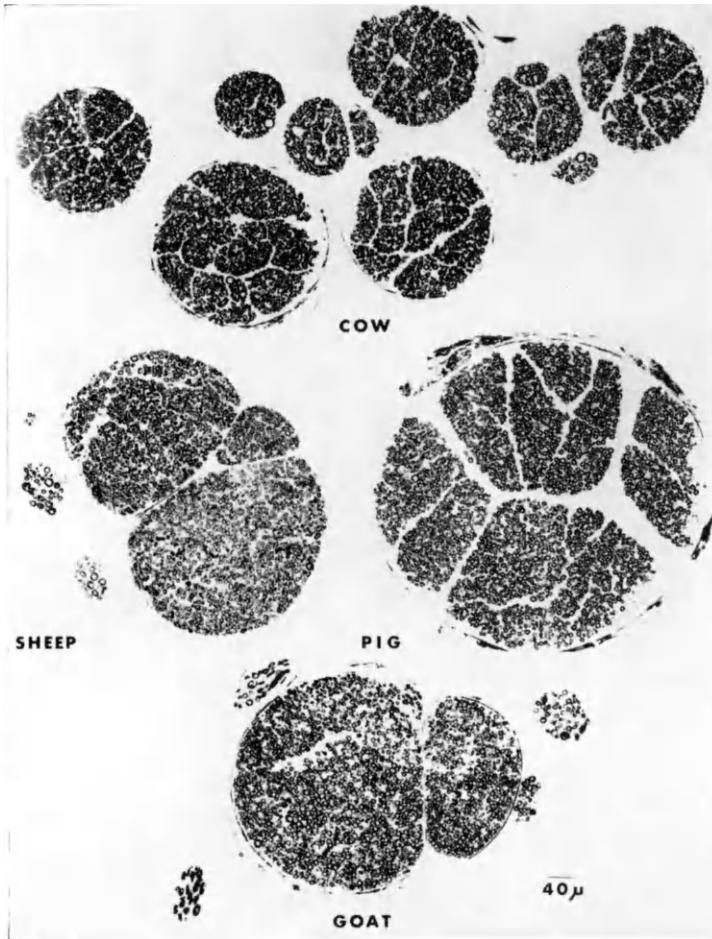
FIG. 6. Composite photomicrographs of cross sections (6μ) of chorda tympani nerves from the cow, pig, sheep, and goat.   Wolters modification of the Weigert-Pal technique.   Original photomicrographs enlarged 750 ×.   Areas of overlap were then removed, the proper pieces assembled and rephotographed. See Fig. 9 for histograms illustrating the fiber diameter frequency distribution of these nerves.   See also Table 1 (Engebretsen and Kitchell, unpublished).

rant of the nerve in a number of interrupted serial sections.   Fibers were seldom sectioned on a tangent in cross-sections of chorda tympani nerves in these animals.   These findings indicate that the fibers in the chorda tympani nerves of dogs and cats are parallel to each other for considerable

distances. The anastomoses between fasciculi of the chorda tympani nerves in pigs, cattle, sheep, goats, and horses makes the isolation of functional "single" fibers more difficult than from the non-fasciculated chorda tympani nerves of dogs and cats.
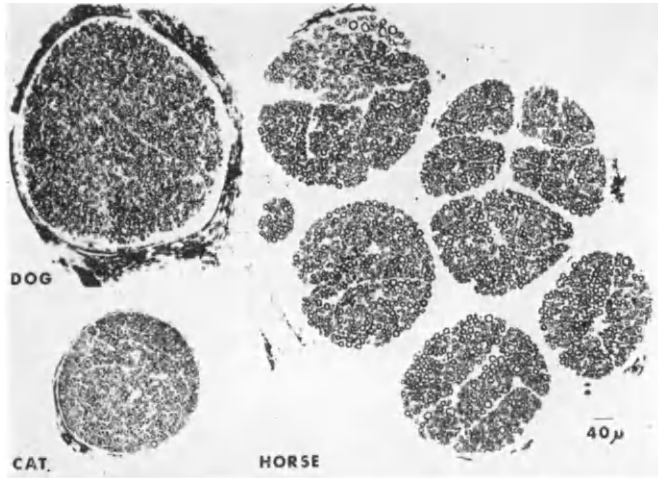
FIG. 7. Composite photomicrograph of cross sections of chorda tympani nerves from the dog, cat, and horse. See legend Fig. 6 for technique. See Fig. 9 for histograms illustrating the fiber diameter frequency distribution of these nerves. See also Table 1 (Engebretsen and Kitchell, unpublished).

The quantity of connective tissue in the chorda tympani nerves was found to be greater in the fasciculated nerves than in the non-fasciculated nerves. The blood vessels within the fasciculated nerves were larger and more numerous than in the non-fasciculated nerves. These anatomical features will be related to technical difficulties in recording neural responses from certain species later in this discussion.

TABLE 1. MYELINATED FIBERS IN THE CHORDA TYMPANI NERVE*

Animal	Average No. of fibers	Fibers less than 6μ	Mode diameter in microns	% total fibers in mode group
Cat	1555	81	4–6	43
Dog	2854	40	6–8	40
Goat	3321	49	6–8	31
Sheep	3423	55	4–6 : 6–8	32 : 32
Pig	4366	53	4–6	27
Cow	5265	35	6–8	31
Horse	5735	37	6–8	38

* Engebretsen and Kitchell, unpublished.

Some of the results of the fiber diameter distribution studies are pre-presented in Figs. 9 and 10 and Table 1. Three nerves from two or three animals of each species were studied. In general, the greater the body mass of a species, the more numerous are the myelinated fibers in the chorda tympani nerve (Table 1). These results are in agreement with similar
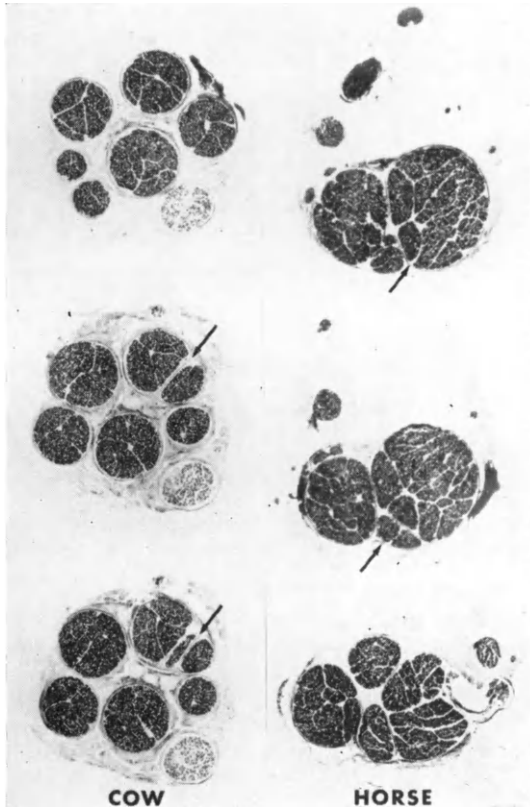
FIG. 8. Photomicrographs of selected cross sections from serially sectioned chorda tympani nerves of the cow and the horse. Arrows point to perineural trabeculae. Note differences in the number of fasciculi at the different levels. Distance between the first and last sections illustrated is within 500μ

results published by Quilliam (1956) in studies of the sural nerve and the nerve to the medial head of the gastrocnemius muscle in several species of animals. Species with greater body mass have more myelinated chorda tympani fibers of larger diameter (Fig. 9). A notable exception to this generalization is in dogs. About 40 per cent of the fibers in the chorda tympani nerves of dogs are less than 6μ in diameter. Goats, pigs, and sheep, each with greater body mass than the dog, have comparatively more fibers

in the chorda tympani nerve of less than 6μ in diameter. The horse and
the cow have, on the average, 10 per cent of the fibers in the chorda tympani
nerve larger than 10μ in diameter. In the goat, pig, sheep, and dog, 3 per
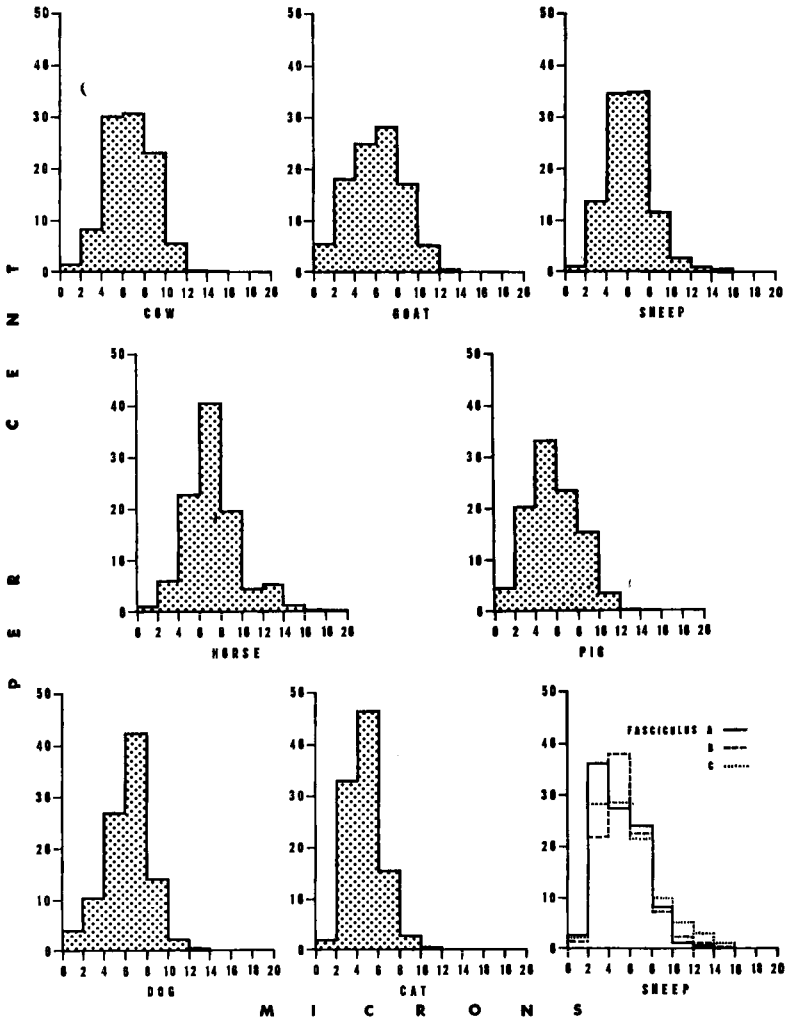
FIG. 9. Histograms of fiber diameter distribution myelinated fibers in chorda
tympani of various animals. See Figs. 6 and 7 for photomicrographs of these
nerves. The superimposed histograms of the fasciculi from a sheep illustrates
fiber diameter distribution in each individual fasciculus. See also Table 1.
(Engebretsen and Kitchell, unpublished).

cent of the myelinated fibers were larger than 10μ in diameter. In the cat
only 1 per cent of the fibers were larger than 10μ in diameter. Analysis of
the fiber diameter distribution by fasciculus in a fasciculated nerve has not

been completed. Preliminary observations indicate that the majority of individual fasciculi contain approximately the same percentage of fibers in each fiber diameter group. Some striking variations to this pattern have
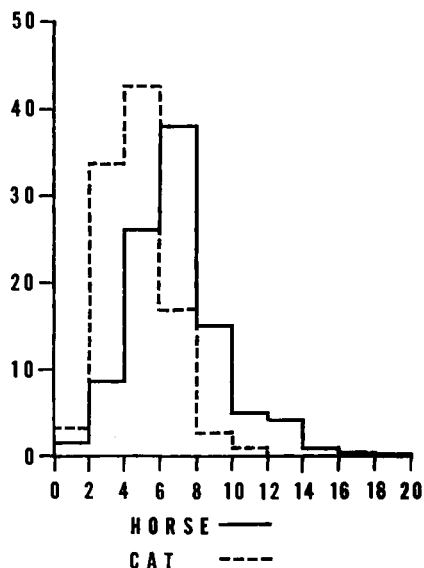
FIG. 10. Superimposed histograms of myelinated fiber diameter distributions of the chorda tympani nerves of the cat and the horse. Each histogram represents the average number of fibers in three nerves. Observe the larger number of larger diameter fibers in the horse. See also Table 1. (Engebretsen and Kitchell, unpublished).

been seen. Some fasciculi contain proportionally large numbers of small fibers and others have substantially larger numbers of large fibers (Fig. 6).

Some of the chorda tympani nerves were prepared, sectioned, and stained using the Holmes silver technique (Holmes, 1947). Quantitative studies of these preparations have not been completed. The number of unmyelinated fibers varies considerably. In Fig. 8 it can be seen that one fasciculus has fewer myelinated fibers in an area than are found in a comparable area in another fasciculus. This fasciculus contains large numbers of unmyelinated fibers. The significance of these variations is unknown.

Previous studies of the chorda tympani nerve in animals are incomplete in respect to fiber diameter distribution. Only the total number of myelinated and unmyelinated fibers has been estimated using various silver staining methods and sampling counting techniques. Foley (1945) estimated that there was an average of 1591 myelinated fibers in the chorda tympani nerve in cats and 2760 myelinated fibers in the chorda tympani nerve in dogs. These estimates are in general agreement with our direct

counts (Table 1). Van Buskirk (1945) estimated that there were 1034 myelinated fibers in the chorda tympani nerve in cats and 1696 fibers in the dog.

The range of the diameters of the myelinated fibers in the chorda tympani nerve of the cat has been estimated to be 1.5 to 6μ, with the majority of fibers estimated to have diameters of 2 to 4μ (Foley and Dubois, 1943). Bruesch (1944) estimated the majority of myelinated fibers were 3μ or less in diameter. Van Buskirk (1945) estimated that myelinated fibers were $2-4\mu$ in diameter. The data presented (Fig. 9 and Table 1) show that some of the myelinated fibers in the chorda tympani nerves in the cat were less than 2μ in diameter. The largest fibers were in the 12 to 14μ diameter group. Andersson, Landgren, Olsson, and Zotterman (1950) estimated that in the chorda tympani nerve of dogs, the myelinated fibers were 4 to 6μ in diameter. The data shown in Fig. 9 and Table 1 illustrate that some of the myelinated fibers in the chorda tympani nerves in the dog were less than 2μ in diameter. The largest fibers were in the 12 to 14μ diameter group.

Sunderland and Roche (1958) studied the axon-myelin relationships in peripheral nerve fibers and have concluded that considerable variation in total fiber diameter, myelin thickness, axon diameter and internode length occurs along individual fibers. They suggest that in a single myelinated fiber the axon diameter is more constant than the total fiber diameter. The results in the present study are not in conflict with their hypothesis. Material prepared in a similar manner from different animals or different species of animals should be subjected to meaningful analysis and comparison.

The neural responses in the chorda tympani and glossopharyngeal nerves have been studied in some of the domestic animals. Figures 11–16 illustrate the results of recent observations. Earlier observations have been published (Kitchell, Ström and Zotterman, 1959 ; Gordon, Kitchell, Ström, and Zotterman, 1959 ; Baldwin, Bell, and Kitchell, 1959). The animal is prepared in the following manner. The animal is deeply anesthetized with a barbiturate anesthetic. The mandible is exposed and resected between the canine and premolar teeth. After ligating the mandibulo-alveolar vessels, the mandible is removed by disarticulation at the temporomandibular articulation. The mucous membranes of the vestibule and the oral cavity are brought into apposition with mattress sutures. The articular disk of the temporomandibular articulation is removed and adjacent blood vessels are ligated. The chorda tympani nerve is located and dissected free from surrounding tissues. A dissecting microscope is used to assist in the removal of fat and as much of the epineurium as possible without damaging the nerve. This " desheathing " operation is relatively simple on the non-fasciculated nerves as found in the dog and cat. In animals possessing fasciculated chorda tympani (Figs. 6 and 7) extreme

care must be taken not to remove or damage small fasciculi lying near the surface of the nerve. After the nerve is properly prepared a " pool " is constructed from the skin and surrounding tissues. Mineral oil is placed in the pool. The temperature of the mineral oil is maintained at 35 to 37°C. The chorda tympani nerve is placed across silver wire electrodes connected to
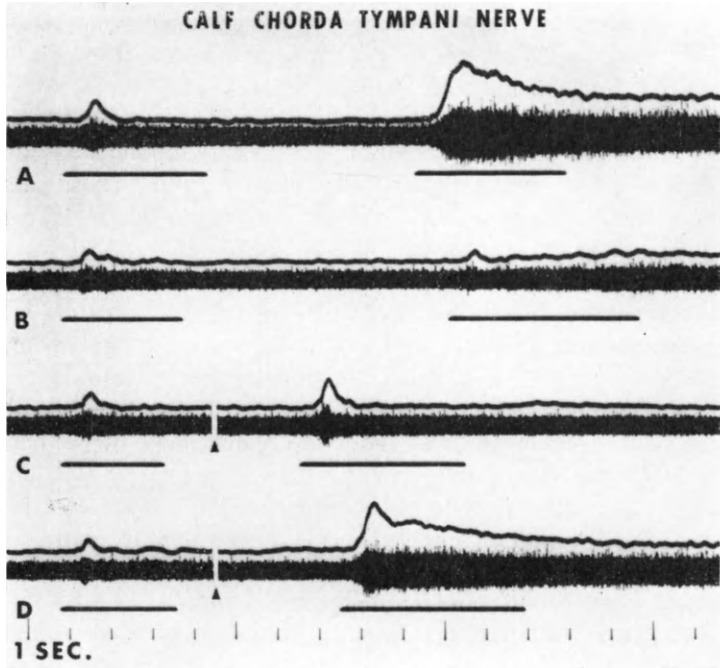
Fig. 11. Records from the whole chorda tympani of a calf. In each are recorded from the top to bottom : The integrated response, the direct response and a signal indicating that the stopcock of the dispensing burette is open. In the last two records a 5 sec segment has been deleted. In each instance the first black signal line indicates the application of distilled water and the second indicates the application of the following solutions. A, 0.5 M sodium chloride. B, 0.46 M sucrose. C, 0.02 M quinine hydrochloride. D, 0.2 M acetic acid (Bell and Kitchell, unpublished).

a Grass P5 low level a.c. preamplifier. The output of the amplifier is displayed on one beam of a dual-beam Dumont oscilloscope for photographic recording. An integrator is connected in parallel to the output of the amplifier. The output of the integrator is displayed on the second beam of the oscilloscope. In certain of the experiments the output of the Grass P5 preamplifier was connected to a Grass 5P3 preamplifier and integrator. The output of the integrator was recorded by the polygraph.

The difficulties encountered in quantitating and interpreting activity in nerves from the tongue has been discussed previously (Zotterman, 1935 ;

Beidler, 1953 ; Kitchell, 1961). Beidler (1953) initiated the use of an integrator as a method of quantifying neural responses in the chorda tympani nerves. The integrator has been proven to be a useful instrument by a number of investigators. It is of considerable importance that during a test series, all other variables are kept as constant as possible (Kitchell, 1961). In making comparisons among species, using whole nerve preparations, consideration must be given to the fact that what is being recorded are electrical phenomena involving recordings from an isolated nerve trunk. For example, in multifasciculated nerves, the amount of epineurium and the quantity of interstitial fluid may markedly alter the size of the recorded potentials. Many factors may influence the direct or integrated response. The magnitude, duration or other characteristics of a response, integrated or otherwise, bears no simple relationship to sensation. This is in no way a denial that certain anatomical features or electrophysiological phenomena do not exist and are not reproducible. The interpretation of these phenomena may lead to serious errors.

In our experiments, the test solution is applied to the tongue by means of a dispensing burette. The quantity and rate of flow of test solution during a series is kept constant. The tip of the burette is adjusted prior to the beginning of a series in order to reduce to a minimum the activation of mechanoreceptors. The lucite flow chamber designed by Beidler (1953) reduces the initial activation of mechanoreceptors but if the concentration gradient changes rapidly some activation of mechanoreceptors due to flow of the solution across the tongue may occur. In our experiments, using a dispensing burette and photographic recording of both the direct action potentials and the integrated response, the phasic response is accentuated. The static response can be analysed by either decreasing the rate of flow from the burette or increasing the quantity of the solution in the burette, or both. The flow chamber has the advantage of exposing a larger surface of the tongue to the test solution than is possible with the dispensing burette, particularly in animals with large tongues. This may account for some of the differences such as concluding that " no " response occurred following the application of a test solution using a dispensing burette while other investigators using a flow chamber state that " slight ", " mild ", or " weak " responses are observed. The flow chamber method is of limited value in studies of phasic responses.

The first step in investigating neural responses in the chorda tympani and glossopharyngeal nerves was to determine if a neural response resulted following the application of distilled water to the tongue. Zotterman (1949) reported a response in the glossopharyngeal nerve in the frog to the application of distilled water (distilled water response) to the tongue. Distilled water responses have been reported in the cat, dog, and pig (Liljestrand and Zotterman, 1954), the rabbit (Zotterman, 1956), the pigeon and

chicken (Kitchell, Ström, and Zotterman, 1959) and the monkey (Gordon, Kitchell, Ström and Zotterman, 1959). These findings have been confirmed in other laboratories (see Fig. 12, Kitchell unpublished ; chickens,
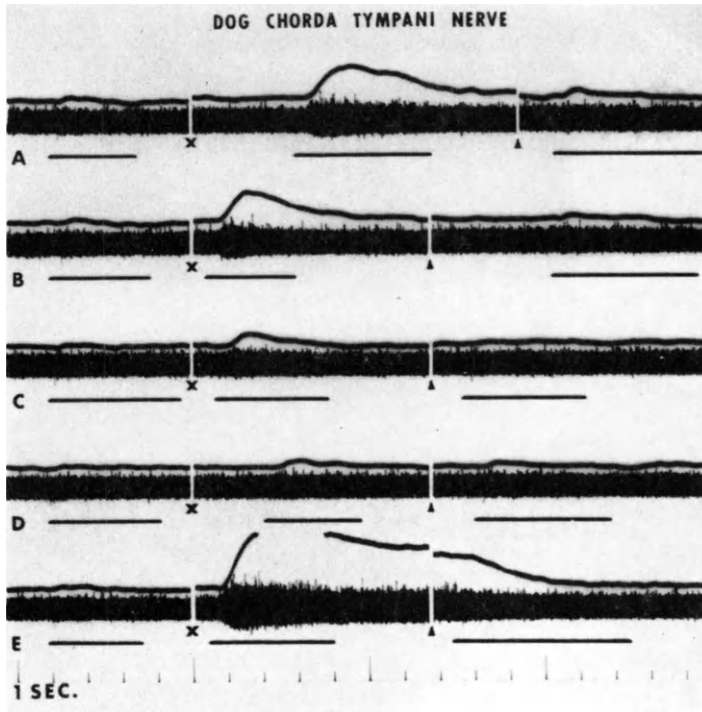
FIG. 12. Records from the whole chorda tympani of a dog. Recording procedure same as in Fig. 11 and 14 except Ringer's solution was applied both before and after the test solution. X indicates 4 sec segment deleted. ▲ indicates 5 sec segment deleted. A, Distilled water. B, 0.005 M sodium chloride. C, 0.05 M sodium chloride. D, 0.1 M sodium chloride. E, 0.5 M sodium chloride (Kitchell, unpublished).

Halpern and Kare, 1961). A neural response to the application of distilled water has not been detected in the rat (Fishman, 1957), sheep, goats, and calves (Baldwin, Bell, and Kitchell, 1959, and Bell and Kitchell, unpublished ; see Fig. 11), and in man (Diamant and Zotterman, 1959). In some animals a distilled water response may be recorded from the chorda tympani nerve but not from the lingual branch of the glossopharyngeal nerve (cat, dog, and rabbit, Appelberg, 1958 ; pig, Kitchell, unpublished, see Fig. 15). The monkey is the only animal studied to date in which a distilled water response has been detected in both nerves (Appelberg, 1958).

The presence or absence of a response to distilled water in the chorda tympani and glossopharyngeal nerves determined whether or not distilled water could be used as the solvent for other test solutions. Liljestrand and Zotterman (1954) observed in animals in which a response to distilled water was present, that if Ringer's solution was applied shortly after the tongue had been washed with Ringer's solution, no neural response followed the second application of Ringer's solution. They recommended using
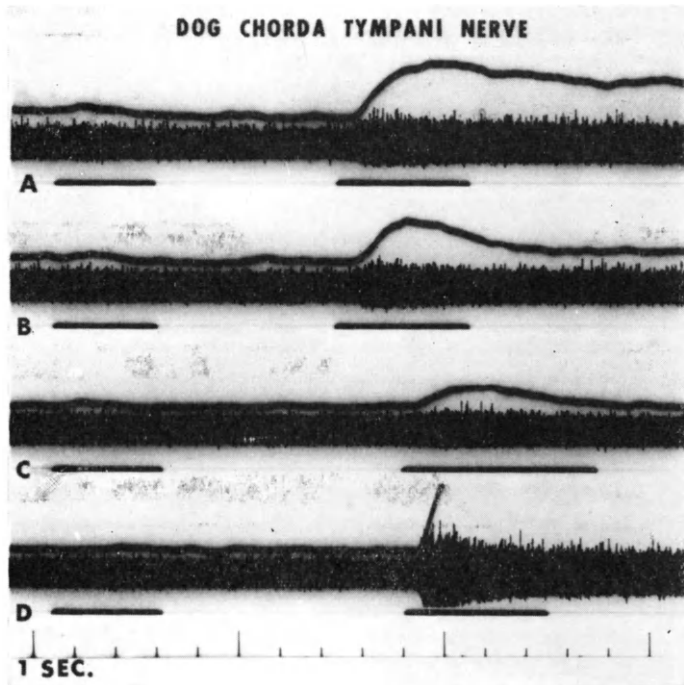
FIG. 13. Records from whole chorda tympani of a dog. Recording procedures same as in Fig. 11. Ringer's solution applied before each test solution. A, 0.5 M sodium chloride. B, 0.46 M sucrose in Ringer's solution. C, 0.02 M quinine hydrochloride in Ringer's solution. D, 0.2 M acetic acid (Kitchell, unpublished).

Ringer's solution as the solvent for the test solutions and the procedure of applying Ringer's solution prior to the application of a test solution to establish a " base line " of spontaneous activity in the nerve. This procedure was followed in the experiments to be discussed here except in those animals where no distilled water response could be elicited. In these animals distilled water was used as the solvent and was applied to the tongue shortly before the test solution was applied.

The test solutions routinely used were 0.5 M sodium chloride, 0.46 M (15 per cent) sucrose, 0.02 M quinine hydrochloride and 0.2 M acetic acid.

The test solutions and the wash (distilled water or Ringer's solution) were maintained at 32–36°C by immersion in a water bath in order to minimize stimulation of thermoreceptors whose axons run in the chorda tympani nerves (Dodt and Zotterman, 1952).

The neural responses recorded from the chorda tympani and lingual branches of the glossopharyngeal nerves following the application of the test solutions are illustrated in Figs. 11 to 16. The gain of the integrator was

FIG. 14. Records from the whole lingual branch of the glossopharyngeal nerve of a goat. Recording procedure same as in Fig. 11 except the region of the tongue where the vallate papillae are located was stroked with a wooden probe twice before and twice or more during the application of the test solutions. Distilled water was applied to the tongue about 10 sec before each record was made. A, Distilled water. B, 0.5 M sodium chloride. C, 0.46 M sucrose. D, 0.02 M quinine hydrochloride. E, 0.2 M acetic acid (Kitchell and Bell, unpublished).

adjusted on the basis of the response obtained in the chorda tympani nerve following the application of the 0.5 M sodium chloride solution. Once a series was started neither the preamplifier nor the integrator settings were adjusted until the series was completed. In most of the animals studied, the integrated response following the application of 0.2 M acetic acid to the tongue was of greater magnitude than the integrated responses elicited by

the other substances. In calves, lambs, and goats the magnitude of response to the application of 0.2 M acetic acid to the tongue was approximately equal or slightly less than the response to 0.5 M sodium chloride.
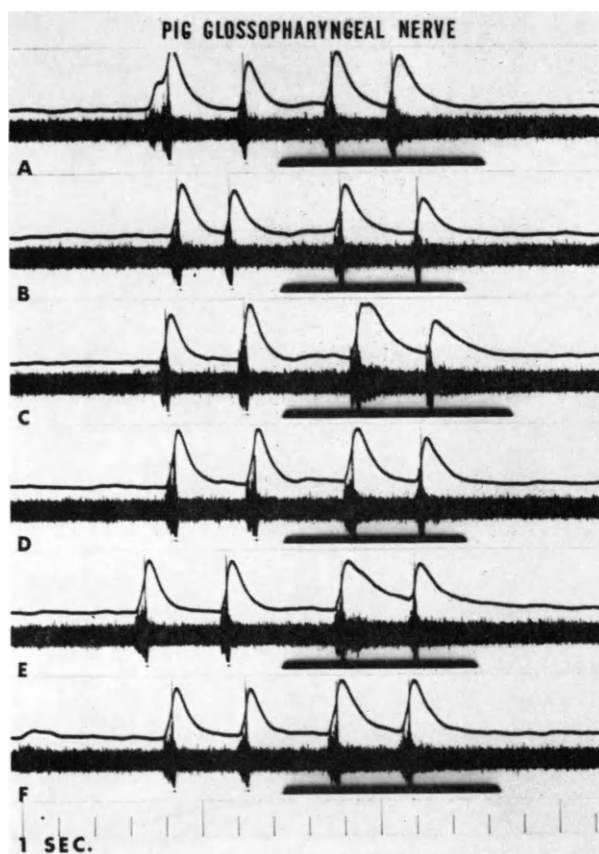
FIG. 15. Records from the whole lingual branch of the glossopharyngeal nerve of a pig. Recording procedure same as for Figs. 13 and 14 except the Ringer's solution was applied to the tongue approximately 10 sec before each record was made. A, Ringer's solution. B, Distilled water. C, 0.46 M sucrose in Ringer's solution. D, 0.5 M sodium chloride in Ringer's solution. E, 0.02 M quinine hydrochloride in Ringer's solution. F, 0.2 M acetic acid in Ringer's solution (Kitchell, unpublished).

In these animals the application of 0.46 M sucrose to the tongue resulted in a response of low magnitude, as recorded by the integrator, but of long duration (Fig. 11). In the dog and the pig, the application of 0.46 M sucrose to the tongue resulted in a response of greater magnitude as compared to the response produced by 0.5 M sodium chloride (Fig. 13).

The application of 0.02 M quinine hydrochloride to the tongue produced

a phasic response in the chorda tympani nerves of calves, lambs, and goats. This substance elicited a relatively larger integrated response in the pig and the dog.
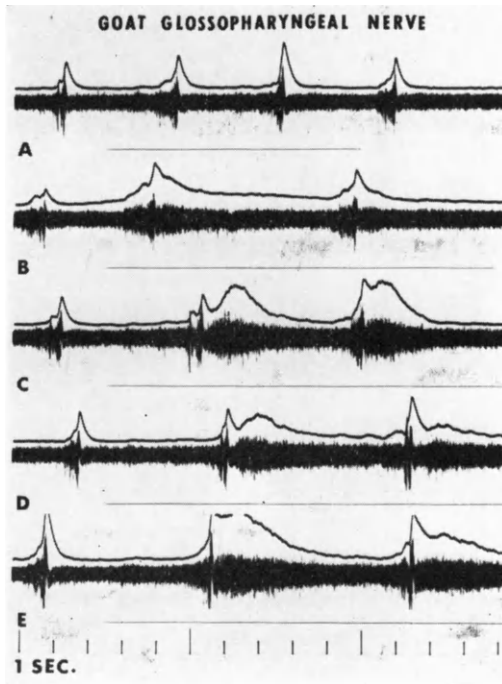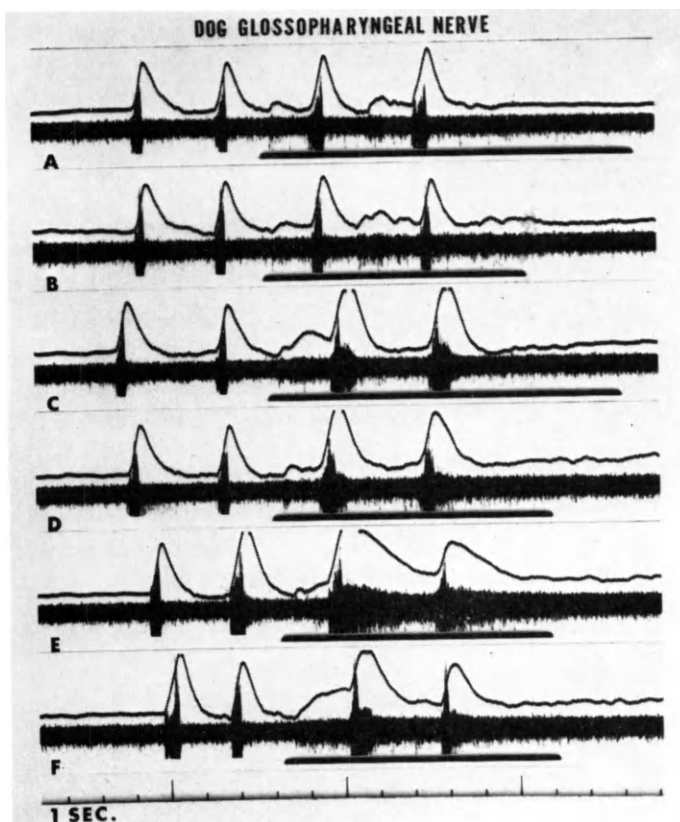

FIG. 16. Records from the whole lingual branch of the glossopharyngeal nerve of a dog. Recording procedure same as for Figs. 11 and 14 except the Ringer's solution was applied to the tongue approximately 10 sec before each record was made. A, Ringer's solution. B, Distilled water. C, 0.46 M sucrose in Ringer's solution. D, 0.5 M sodium chloride. E, 0.02 M quinine hydrochloride in Ringer's solution. F, 0.2 M acetic acid in Ringer's solution (Kitchell, unpublished).

The neural responses recorded in the lingual branch of the glossopharyngeal nerve following the application of the 4 test solutions is illustrated in Figs. 14–16.   Mechanical stimulation of the tongue near the vallate papillae produced neural responses of larger magnitude than were obtained in the chorda tympani nerves using similar methods of stimulation. The rate of flow of the solutions from the burette had to be carefully adjusted to minimize stimulation of mechanoreceptors.   The flow of test

solutions over the caudal part of the tongue often did not elicit in a response (Figs. 14 and 15). Movement of a vallate papilla regularly elicited a phasic response (Fig. 14A). If a test solution was flowing over the papillae when they were being moved the response to mechanical stimulation was followed by a second response of longer duration. This phenomenon was first reported by Appelberg (1958) and was related to the fact that the taste buds associated with the vallate panillae are located near the base of the moat which surrounds the papillae. He presented the hypothesis that movement of the papilla opened the moat and thereby permitted the test solutions to contact the taste buds. Our results have confirmed his observations (Figs. 14–16). Movement of a vallate papilla during the application of distilled water or Ringer's solution did not elicit a detectable secondary discharge which could be related to the application of these solutions (Figs. 14–16). Striking secondary responses were observed in goats during the time the various test solutions were being applied (Fig. 14). The magnitude of the integrated responses which followed the application of 0.46 M sucrose and of 0.02 M quinine hydrochloride were of interest considering the low magnitude of integrated response observed in the chorda tympani nerve following application of these test substances to the rostral areas of the tongue in goat.

Results obtained in the pig (Fig. 15) indicate that the neural responses in the lingual branch of the glossopharyngeal nerve following the application of 0.5 M sodium chloride and 0.2 M acetic acid to the caudal part of the tongue while moving the vallate papillae are smaller than those following the application of 0.46 M sucrose and 0.02 M quinine hydrochloride. Further studies in this species are contemplated.

In conclusion, comparative anatomical and physiological observations of gustatory mechanisms have been presented. In particular the myelinated fiber diameter spectrum of the chorda tympani nerve in several domestic animals has been presented. Neural responses recorded from the chorda tympani and the lingual branch of the glossopharyngeal nerves following the application of various test solutions has been described. The need for caution in arriving at conclusions based upon comparative phenomenal events is emphasized. This does not preclude the importance of recording and describing phenomena but only emphasizes the need for considerate and thoughtful judgements before generalizations from specific phenomena are made.

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TASTE STIMULATION AND PREFERENCE BEHAVIOR*

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ONE intriguing aspect of the chemical senses is the potent control of behavior which they effect in invertebrates and vertebrates, including mammalian forms. The almost ubiquitous " sweet tooth ", the preference for sugars in many but not all species, and the compensatory taste cravings which appear in states of physiological need or endocrine imbalance are well known, the latter particularly from the work of Richter (1942) and subsequent workers. A number of investigators have examined both taste sensitivity and taste preference in an effort to determine the relative roles of the gustatory stimulus itself, post-ingestional factors, and learning effects. In our work, we have looked particularly at certain features of the afferent input and its central neural mechanism in relation to behavior, the so-called preference and aversion responses for different taste stimuli displayed by the laboratory mammal, primarily the rat.

Especially dramatic is the enhanced intake and preference for NaCl following induction of physiological need by adrenalectomy, dietary reduction of NaCl, or other physiological manipulation. And, although there has been much work on the subject, there is still debate as to the precise mechanism by which the specific hunger for sodium chloride is mediated. Several noteworthy points may be mentioned.

1. The normal rat, apparently well supplied with NaCl, displays a preference for hypotonic and isotonic solutions and an aversion to hypertonic salt solutions.

2. Adrenalectomized or deprived rats show an enhanced intake or preference at all concentrations with the result that the preference threshold is lowered, i.e. animals show a preference for weak solutions at a concentration below those taken by normal animals and they take more of the stronger suprathreshold solutions.

3. The preference for sodium salts in the sodium deprived animal is highly specific; NaCl is taken in preference to KCl, NH_4Cl, $CaCl_2$, or other taste stimuli.

4. In many situations, the preferential response to NaCl after deprivation is immediate and thus appears to depend on some change in the taste or response to the taste of the stimulus without prior learning.

5. Efforts to assess the locus or nature of these changes, whether in peripheral receptor sensitivity or in central neural mechanisms, point to central processes.

Our early studies of sensitivity (Pfaffmann and Bare, 1950) indicated that the receptor threshold for NaCl in adrenalectomized rats was not different from normal and that the receptor threshold itself fell considerably below the behavior preference threshold of normal animals. Since that time, conditioning studies in rats (Carr, 1952 ; Harriman and MacLeod 1953) have confirmed the fact that thresholds of normal and adrenalectomized rats are not different and Koh and Teitelbaum (1961) have recently shown a general correspondence between the thresholds obtained by conditioning methods and those observed electrophysiologically. But, since our early recording studies were concerned only with threshold values, Dr. Marvin Nachman* (1962b) and I have recently re-examined the chorda tympani responses in salt deprived animals over a wide range of NaCl concentrations and for a number of other suprathreshold stimuli. In particular, we wished to determine whether relative changes occurred in the response to NaCl compared to other salts and taste stimuli.

In place of adrenalectomy, we employed a salt deficient diet for a 20-day period, half of the animals on the diet being used in behavioral tests to show that an enhanced NaCl preference had been established. A two-bottle, 2 hr test was used in which each animal was pre-trained to sample and drink from two drinking tubes mounted on the front of a small individual cage. Six rats in each group, experimental and control, were pretrained and then tested while 22 hr thirsty for the preference between distilled water and 0.4 M NaCl solution. This salt concentration is quite aversive to the normal rat.

Figure 1 shows the mean cumulative intakes in the test and indicates that the control animals took very little salt and mostly water, whereas the deprived rats in the first 15 min took more salt than water. Later in the period, the intake of water by experimental animals showed a significant rise whereas salt drinking continued at a reasonably regular rate. This late rise in water intake probably reflects a post-ingestive or thirst effect produced by the hypertonic salt. Although the method of plotting exaggerates this late rise, it is equally evident with a linear time scale. Close examination of the early portions of the water and salt curves indicates that the first acceleration of water drinking occurs at about 5 min, which could mean that this is the first sign of an immediate post-ingestive effect perhaps different from the later thirst effect. Six other rats were tested for

*Special Public Health Service Postdoctoral Fellow.

their preference between 0.1 M NaCl and 0.1 M KCl after pre-training on water in the test cages, but in these tests they were not thirsty. All three sodium deficient S's, immediately and relatively continuously, ingested large quantities of 0.1 M NaCl (a mean intake of 44.2 ml at the end of 2 hr) compared to very little KCl (a mean of 0.4 ml). The non-deficient rats took 4.1 ml of NaCl and 0.4 ml of KCl. Thus, the deprivation schedule induced a behaviorally significant NaCl preference.
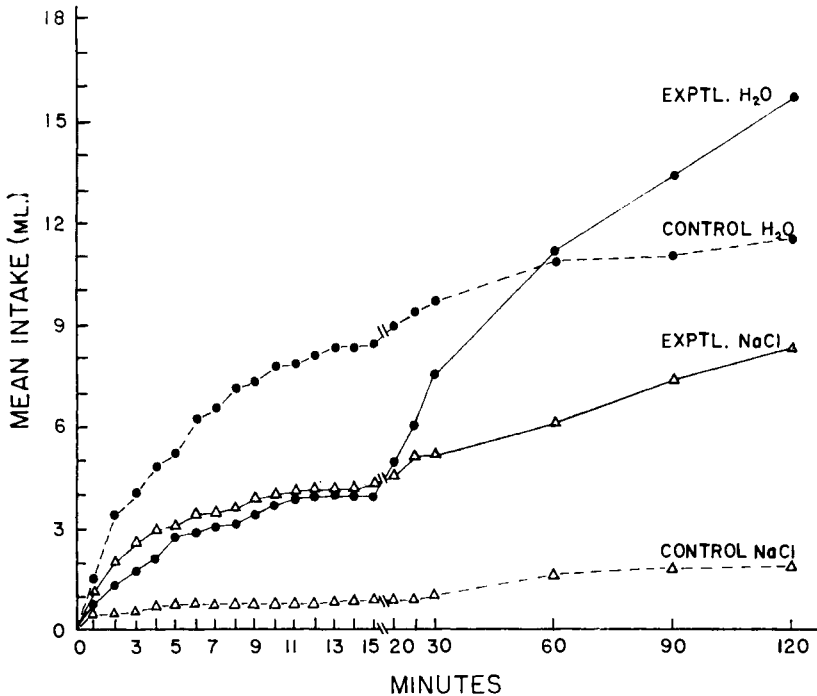
FIG. 1. Mean cumulative intake of water and 0.4 M NaCl solutions by control and salt deprived rats.

The electrophysiological measure from the whole chorda tympani nerve was obtained on a second group of animals (6 controls and 6 experimentals) given the same diets but no behavioral tests which would interfere with their salt deficiency. Our earlier study (Pfaffmann and Bare, 1950) has sometimes been criticized because the adrenalectomized rats had been maintained on 3 per cent NaCl solution from the time of adrenalectomy to recording. The control and experimental diets were identical except that 1 per cent NaCl was added and mixed with the salt-free food. The nerve was exposed in pentobarbital anesthetized animals using our usual ventral approach, which provides a natural moist chamber and maintains the nerve in good condition. For purposes of quantitative comparisons between animals,

the magnitude of deflection as recorded with summator and inkwriter was expressed as a ratio of the response to 0.01 M NaCl. In many preparations, however, the same absolute level of response was obtained. When higher amplification was required, this could be attributed to incidental features such as trauma to the nerve, variation in inter-electrode spacing, variations in moisture, and shunting of the electrodes, etc.

No systematic differences between experimentals and controls were observed either in absolute or relative magnitudes of response to the supra-threshold stimuli, NaCl, NaAc, KCl, NH_4Cl, $CaCl_2$, all at 0.1 M, or 0.1 M quinine, 1.0 M sucrose, or 0.003 M HCl. Figure 2 compares the responses to a series of NaCl concentrations. These functions are the same for both
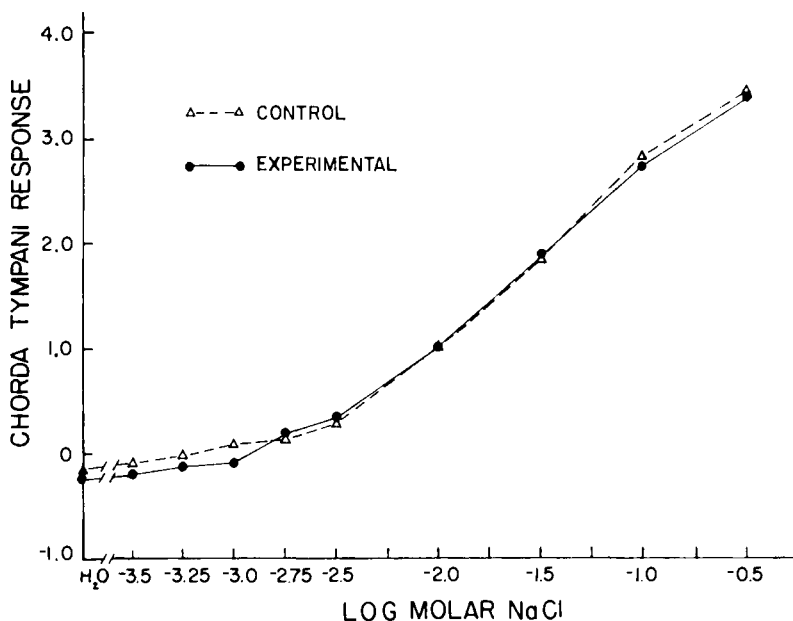
Fig. 2. The magnitude of chorda tympani responses to NaCl solutions in normal and NaCl-deprived rats.

experimentals and controls and are very similar to those reported for the rat earlier by Beidler (1953) and by Pfaffmann (1955). In our previous accounts, we used one-minute intervals of stimulation and 1-min intervals between stimuli ; here we used 3-min intervals, which show more clearly the decrement in resting activity upon the application of H_2O or weak saline solutions (see Fig. 3). Zotterman (1956) made similar observations in his studies of the water receptor in different species. The rat does not give a water response except as an off-type discharge following sucrose or HCl stimulation. In other species, the water response is influenced by

prior stimulation or adaptation by other ions.   Perhaps the fluid electrolyte balance in the rat maintains the receptors in an inhibited state for water stimulation as compared with other species.   The lesser decrement to weak salts is systematic with concentration so that a neutral point (no change in
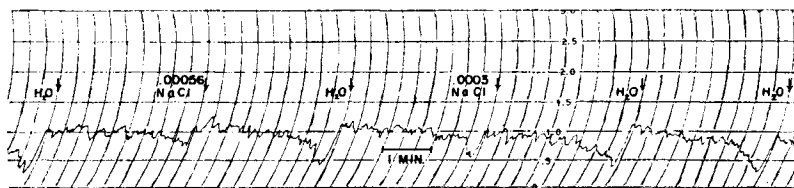
FIG. 3.   Summator record of chorda tympani responses to water and weak salt solutions.   Water reduces the level of activity; weak saline solutions cause less reduction.   Stronger concentrations above 0.0018 M produce increments in discharge.

resting activity) is observed at about 0.001 M NaCl.   Concentrations above this level give the usual increment in discharge ; below, they produce a decrement but the exact point where this change occurs depends upon prior stimulation and the interval between stimulations.

The above results confirm our early observations at threshold and extends them to suprathreshold levels of NaCl and other salts and taste stimuli.   It must be remembered that these animals were surgically anesthetized and so these observations do not rule out the possibility of afferent modulation by reticular influences or by other neural efferents.   Further examination of this possibility is needed.

We do have evidence of receptor modulation by another factor, namely, level of salivary electrolytes.   Our recording results argue against any intrinsic chemical change in receptor mechanism, yet the recent studies of Yensen (1959), de Wardener and Herxheimer (1957), and Henkin and Solomon (1960) all report changes in threshold with changes in fluid intake or salt deprivation.   McBurney* and I (1962) have recently made threshold determinations for NaCl under conditions which approximated those we employed in the electrophysiological studies, that is, when the tongue was well rinsed with water and free of all saliva as compared with the tongue bathed with saliva between stimulations.

McBurney devised a continuous flow system resembling that used by Hahn (1934) a number of years ago but without the flow chamber.   The subject sat with his tongue slightly extended under a spout which delivered a steady stream of distilled water.   Upon a signal from the experimenter, the subject moved his tongue aside from the rinsing stream so that 10 ml of taste stimulus could be presented by a pipette to the anterior surface of the tongue.   The subject then returned his tongue to the rinsing stream until

*On a Fellowship grant from the General Foods Corporation.

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the next stimulus. Solutions were maintained at about 34°C, which was neutral for the arousal of temperature sensations in our situation. We found that, depending upon the subject, the threshold for detection of NaCl was 100 to 1000 times lower under the continuous rinse than when the tongue was bathed by saliva between stimulations. This great increase in sensitivity with adaptation to distilled water suggests that salivary components provide an ambient adapting background, perhaps akin to the effect of general illumination on visual dark or light adaptation. These results recall to mind those obtained by Hahn a number of years ago with his *Geschmackslupe*, a flow chamber stimulator. This advice adapted the tongue to distilled water or solution and restricted the stimulus to a circumscribed area. One of his subjects gave a threshold for NaCl of 52×10^{-4} M, whereas a second gave a value of 13×10^{-9} M (Hahn, 1949). The extremely low value of the second subject is lower than we have seen in our most sensitive subject.

Hahn showed that adaptation to different concentrations of NaCl elevated the threshold for NaCl to a value just above that of the adapting stimulus. Most of his stimuli were well above threshold. Accordingly, McBurney examined the effect of adapting the tongue to weak salts closer to threshold concentrations using 0.000069 M, 0.00069 M, and 0.015 M NaCl, as well as distilled water and saliva. Figure 4 illustrates the change in threshold under all these conditions for one subject. Four subjects in all were employed and each showed the same effect. The figure shows the concentration of adapting solution along the abscissa and concentrations of threshold for detection determined by a modified ascending method of limits. " Check stimuli " of pure water were introduced randomly in the stimulus series and false reports to water were corrected. This maintained a false alarm rate for four subjects at about 20 per cent. A fifth subject's results were discarded because of an excessive false alarm rate.

Two conditions of rinse or no rinse were obtained on any one day in a partially counterbalanced manner so that 6 days of experimentation were required to obtain 12 data points. The diagonal line shows the iso-equality values for adapting stimuli and threshold. Threshold values greater than the concentrations of the adapting concentrations fall above the diagonal line. The value of the salivary sodium for each S, determined by a flame photometer analysis, is plotted at the appropriate abscissa value. Each point is the threshold for one daily session.

These results indicate that the threshold is significantly altered by adaptation to water or weak saline solutions. Furthermore, the threshold values obtained when the tongue is adapted to saliva closely approximates the value to be expected if salivary NaCl were the primary factor influencing NaCl thresholds. These results emphasize the importance of giving due regard to the influence of ambient salivary background. It would be

expected that not only thresholds for saline but thresholds for many other stimuli might be similarly dependent upon salivary composition. Indeed, Cragg (1937) a number of years ago showed that salivary pH was related to threshold for acids. Our observations extend this concept to salt sensitivity and further suggest that a simple process of adaptation may account for the effect.
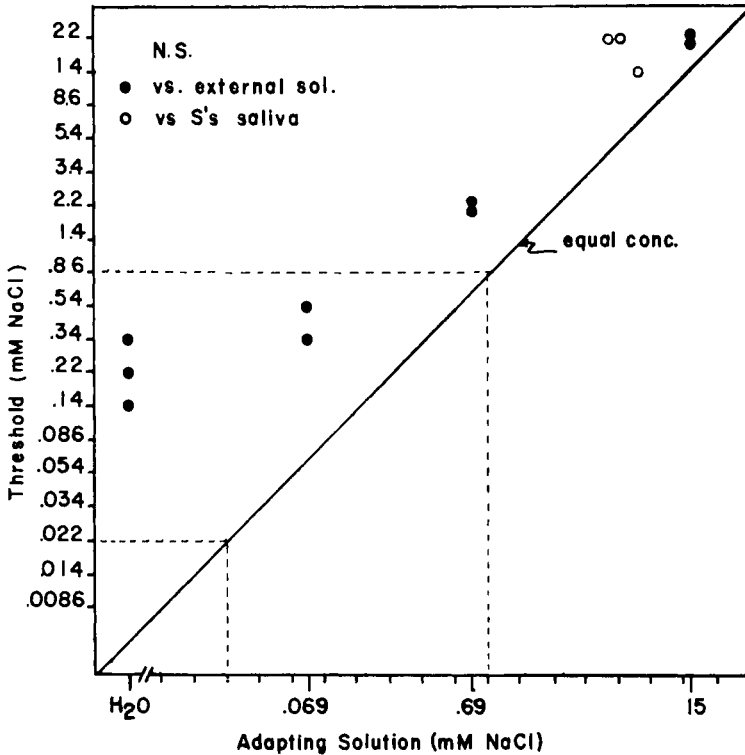
FIG. 4. The relation between adapting solution on the tongue and threshold. Each point shows the threshold obtained on one experimental determination; solid dots for adaptation to water or salt, open circles where the tongue is adopted to saliva with a Na+ composition shown on the abscissa as determined by flame photometry.

It should be noted that when adapted to the salts used in our studies, S's reported *no* salty sensations at all, even for the strongest stimulus. Yet, when adapted to water, these same stimuli elicited strong sensations of saltiness. At the end of the experiment, the change in taste due to adaptation was demonstrated to each S. Each was genuinely surprised at the effect.

The magnitude of the changes in sensitivity induced by adaptation lie clearly within the order of changes reported by Yensen and others when

sodium need has been manipulated in man. Since salivary sodium levels and salivary volume flow are sensitive to changes in serum Na and hydration it is entirely possible that such threshold changes reflect changes in salivary sodium and other electrolyte constituents. Thus, the electrophysiological evidence just reviewed for chemical stability of receptor sensitivity can be reconciled with the psychophysical studies on man.

The question still remains on the relation between taste sensitivity and preference behavior motivated by salt deficiency. Changes in sensitivity, however induced, might increase the detectability of taste stimuli but there is no necessary relation between enhanced sensitivity and preference. It should be remembered that preferences in salt needy animals are enhanced over the whole range of concentrations tested. Mere change in sensitivity should increase the apparent strength of the higher but more aversive salt stimuli. It is just these solutions that are taken with increased avidity.

Another way to induce salt need is by dialysis with 5 per cent glucose injected intraperitoneally in an amount equal to 10 per cent of the animal's body weight. This draws NaCl into the body cavity as osmotic equilibrium is established and, if this ascitic fluid is withdrawn after several hours by a second intraperitoneal puncture, the animal is left in a highly depleted sodium state. This results in increased drinking of sodium chloride solutions and of water (Falk and Herman, 1961). In a single-bottle drinking test, the dialyzed animal takes increasing amounts of salt solution, depending upon concentration, and shows an exaggerated preference-aversion function (Richman and Pfaffmann, 1962).

Behavior in a two-bottle preference test is likewise altered. We have studied this in a new version of the two-bottle preference test developed by Dr. G. L. Fisher (1962) in our laboratory. The drinking tubes are presented alternately once a minute by a solenoid operated plunger so that only one tube at a time is available to the animal. Tests are conducted for 20 min just after the animal has been fed and watered, at the same time each day. An electronic record of drinking is obtained by a Grason-Stadler drinkometer and cumulative recorder which advances the pen at each lick. At the end of each, the recorder resets to zero as the one tube retracts and the other is presented.

Figure 5 shows the last session before and the first session after dialysis. In the left record, the signal marker up means that tube no. 2, containing water, is available ; signal marker down, that tube no. 1, containing salt, is available. In the right figure, tube no. 1 contains H_2O and no. 2, salt. Before dialysis, a slight preference for saline is shown at the beginning of the session, but the animal drinks little thereafter. After dialysis, much greater drinking of salt ensues. Water is sampled but not consumed to any degree, as shown by the square-topped drinking records. Not only is there increased ingestion of salt, but there is a clear preference, after sampling,

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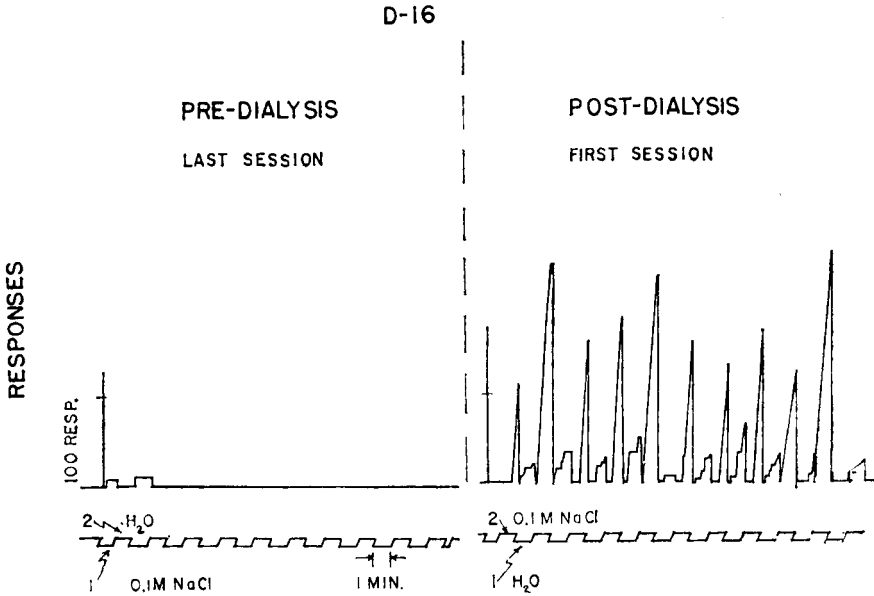
FIG. 5. Drinking behavior before and after inductions of Na need by dialysis; apparatus and techniques described in text.

for the saline. Figure 6 shows the tabulation of licks for each of the two solutions for a series of test days preceding and following a single dialysis. On 5 of the 7 days before dialysis, the rat selected NaCl solutions and on

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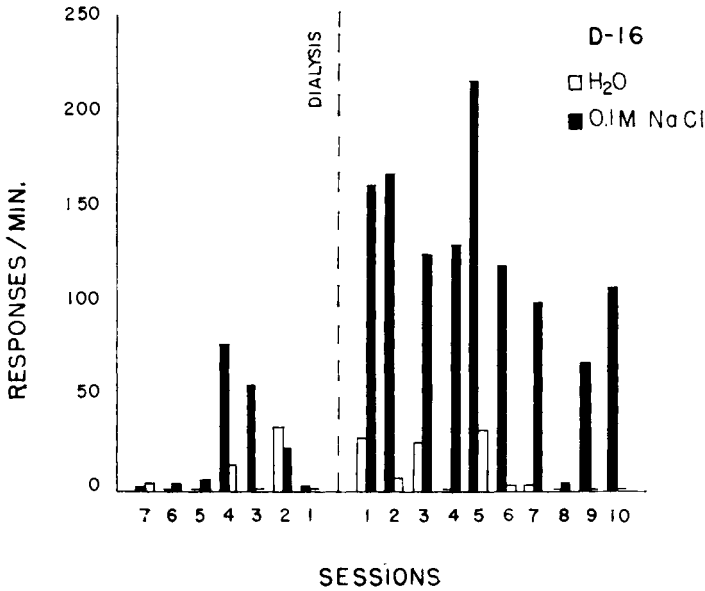
FIG. 6. The preference for NaCl on 7 days before and 10 days after dialysis.

2 days it selected water. On every one of the 10 days post dialysis, the animal preferred NaCl and ingested large quantities of it. It is of interest that the preference for salt was still apparent on the tenth day, long after the major disequilibrium in Na metabolism had presumably been corrected. This animal continued to show an enhanced salt preference for 34 days after dialysis. This points to the importance of a single need-reducing experience in enhancing the preference value of a taste stimulus,

The selectivity of the preference for NaCl is shown in Fig. 7, where 0.1 NaCl was paired with 0.1 KCl. Note the greatly enhanced intake and preference for NaCl after dialysis and the decreasing intake of KCl in successive

FIG. 7. Record of preference behavior for NaCl over KCl after dialysis.

1-min periods which resembles an extinction series as the animal comes to make a clearer discrimination between the two salts.

Further data on the question of specificity for taste of different salts and on the effect of post-ingestion factors is provided by a recent study Dr. Nachman just completed while a guest investigator in our laboratory. Nachman (1962a), using his 10-min two-bottle test, with pre-training to insure sampling from both tubes, observed that naive adrenalectomized rats showed an equal preference for NaCl and LiCl. In the same test, sodium solution was readily preferred to K, NH_4, or Ca solutions. On the other hand, Fregley (1958) reported that rats avoid LiCl solutions in the 24-hr test. Lithium salts have been shown to be toxic to a variety of animals. In dogs, the major symptoms were a marked diuresis, tremor,

salivation, lethargy, anorexia, weight loss, muscular weakness, and death. Lithium chloride was, for a while, marketed as a salt flavoring substitute for persons on a low sodium diet until several cases of severe poisoning were reported.   In rats, the toxic after-effects apparently occur rapidly since the rats stopped drinking during a 10-min period and soon became lethargic and extremely inactive so that they lay flat on the floor of the cage.

Beidler (1953) and Fishman (1957) showed that Li and Na produced very similar amounts of activity in the chorda tympani and that in single fiber studies the discharge produced by Na was similar to that by Li. Thus, it would appear that the taste of the two salts is highly similar and leads to acceptance of either in short tests but rejection of Li after the post-ingestive toxicity takes effect.   Nachman also showed that the learned aversion to Li generalizes most to NaCl, next to NH_4Cl, and least to KCl, which follows the order of magnitude of size of chorda tympani response.   Erickson (1962) has recently demonstrated that rates of discharge of single units in rat chorda tympani for KCl and NH_4Cl were highly correlated, whereas no systematic relation held between these two salts and NaCl discharges.   Similarity of chorda tympani patterning presumably signifies similarity of taste.   These results are in accord with Nachman's generalization data showing that rats can readily discriminate the taste of either NH_4Cl or KCl from that of LiCl and NaCl, while they apparently have great difficulty in discriminating NaCl from LiCl.

Two points bear emphasis in these experiments.   First is the immediacy of the enhanced preference for lithium and sodium in the initial post-adrenalectomy tests in animals that have never been exposed to salt solution prior to the test.   This implies that there is an immediate effect of need state upon the taste of salt, which also generalizes depending on the degree of similarity to the taste of sodium chloride.   Secondly, the learned aversion to lithium persists for many months unless extinction training is used.   This shows the importance of a single post-ingestive effect on changing the value of a taste stimulus.   The prolonged enhanced preference for salt after dialysis described above also leads to a similar conclusion.   These and other studies point to two aspects of taste preference in states of need that have often been treated as antithetical, e.g. the direct operation of taste factors versus the delayed operation of post-ingestive metabolic consequences.   Both can be shown to be important and both, presumably, can interact to varying degrees under different conditions.

Two recent studies have emphasized the role of water metabolism and hydration in the preference-aversion function for salt solutions in the non-deprived animal.   Deutsch and Jones (1960) found that mildly thirsty rats learned to run to the arm of a T-maze that contained water, rather

than the other arm which contained salt solution. Under these conditions, the rat seemed motivated for water. A two-bottle preference test for the rat with the same degree of deprivation, however, showed the usual preference for salt over water. They believe that, in both cases, the mildly thirsty animal desires water, not salt, but that in the drinking test he is fooled by the taste of weak saline. Normally, the spontaneous discharge of the taste receptor in the rat is reduced by water. Weak saline solutions are less effective than water in reducing the resting activity, as shown in Fig. 3. The smaller decrement to salt is said to be a "less good" water signal and the rat must drink more saline to achieve an equivalent afferent signal to water.

As we shall see below, the preference for saline solutions as displayed in the two-bottle test (24-hr *ad lib.*) may be regarded as a learned response to the extent that the rat overcomes position habits in that situation and learns where and how to find the salt which he prefers. When the 23-hr thirsty animal is placed in a two-bottle situation, he drinks indiscriminately (Treidman and Pfaffmann, 1961). If intake is followed minute by minute in a 20-hr deprived rat, indiscriminate drinking of water and salt occurs at first, but after 10 min of drinking, an increased intake of saline occurs and the rat begins to display the preference for salt. At the end of a 20 to 30 min drinking test, the deprived animal will show a "salt preference" in terms of final total fluid consumed, whereas there may be no salt preference in the early minutes of drinking (Nachman, 1962b). In the T-maze, on the other hand, the animal never drinks enough water (or saline solution) to satisfy his thirst and, therefore, he remains thirsty throughout the training and learns to go to water. Other evidence by Mook (1962) tends to show that saline solutions are less effective for hydrating a deprived animal than plain water. Thus, the acquisition of a response to the water side of the T-maze is to be expected. The Deutsch and Jones results in the maze may depend primarily on thirst drive, whereas the preference tests in the same animals are influenced by both thirst plus the salt preference. The maze and preference test are two different situations.

Dr. Fisher's preference testing device with the alternate presentation of water and salt was used to monitor the development of preference responses to water and 0.1 M NaCl continuously over several 24-hr *ad lib.* sessions. The positions of the salt and water were interchanged every 12 hr. In the first 12 to 24 hr, all animals showed varying degrees of water and/or salt intake but gradually, as the animals gained more experience with the situation, they built up a stable and reliable preference for salt. In the beginning, the animals seem to drink steadily at the first tube encountered ; in some cases a position habit was evident. As time progressed, sampling at both tubes became apparent and at a critical stage, usually late in the
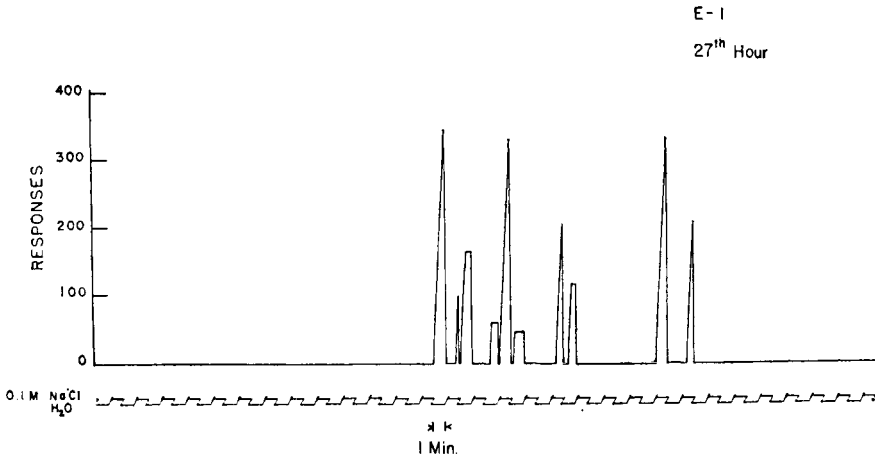
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27th Hour

FIG. 8. Record of development of preferential drinking of NaCl in a continuous *ad lib.* situation.

first or early in the second day, alternate sampling and preferential choice of saline was clearly observed. Figure 8 illustrates one such transition period, after which the animal displayed a stable salt preference. Table 1 shows the number of licks at salt and water tubes from the beginning of the observations for one animal.

TABLE 1. RAT NO. 2, NUMBER OF LICKS

Periods of 12 hours		Distilled H$_2$O	Position	NaCl	Position
Day 1	1	1252	1	14	2
	2	29	2	7054	1
Day 2	3	2148	2	32	1
	4	189	1	6199	2
Day 3	5	23	1	3687	2
	6	73	2	8359	1
Day 4	7	134	2	3796	1
	8	122	1	4914	2

After the first day, the animal showed a consistent preference regardless of the position of the tube containing salt (1 = R, 2 = L). Examination of the continuous recording showed that occasional sampling of " the other " tube continues throughout but that, when water was encountered, drinking stopped and the animal waited or shifted to the saline container. This behavior is clearly preferential, not just the chance drinking of

" dilute water ". If the animal wants water, it is hard to see why he stops drinking water in order to take saline, the less good or more " dilute water ". Other evidence by Young and Falk (1956) and Falk (1961) also points to the fact that the salt preference can reflect itself even in loco-motor responses, i.e. preferential running to saline. All these results are incompatible with the Deutsch and Jones formulation.

Mook's (1962) ingenious " electronic esophagus " makes it possible for the experimenter to deliver the same or different solutions through an entubed gastric fistula while the animal drinks. The fluid which the animal drinks escapes through the esophageal fistula but, since a drinko-meter monitors the rate of licking, appropriate volumes of test fluid may be introduced into the stomach by a solenoid pump system. If the stomach receives the same solution that the animal drinks, then the normal preference-aversion functions are obtained for NaCl, sucrose, and glucose. Instead, if pure water is intubed, then the response to these three solutions is markedly changed : NaCl no longer yields a preference-aversion function ; sucrose and glucose yield functions with concentration which resembles the chorda tympani discharge function for sugar as described by Hagstrom and Pfaffmann (1959). Mook concludes that in normal animals salt drinking is largely a function of hydration and hence not determined by taste. The sugar responses are clearly more taste determined. Mook, however, points out that his data refer to a single-bottle intake situation in a somewhat *thirsty* animal. This would tend to maximize the hydration, post-ingestion effects. Desensitization by deafferenting the tongue or ablating the primary gustatory relay of the thalamus disrupts the usual preference-aversion behavior in the otherwise normal animal.

The animal can drink both solutions but has no sensory cue. Both preference to salt and sugar as well as the aversion to quinine were markedly reduced by lesions in the medial tip of the ventro-basal tip of the rat's thala-mus (Ables and Benjamin, 1960 ; Oakley and Pfaffmann, 1962). Figures 9 and 10 show the great reduction in preferences to both salt and sugar we observed. The areas ablated correspond to the regions from which electrical activity may be recorded (Pfaffmann, Erickson, Frommer, and Halpern, 1961 ; Emmers, Benjamin and Blomquist, 1962 ; Benjamin, 1962). If post-ingestional factors alone were responsible for the behavior, we should expect ageusia or hypogeusia to have much less effect on intake than they actually have.

There is still another class of behavioral methods, the operant procedures developed by B. F. Skinner (1938) that can be used to assess the role of taste stimuli in controlling behavior. Many types of taste solution may be used as positive reinforcers for a bar pressing habit. The particular utility of these methods for taste studies is that the experimenters can maximize taste and minimize post-ingestional effects by using appropriate schedules

of intermittent reinforcement (variable intervals or ratios, depending on the schedule). The smaller the volume and the less frequent the reinforcement, the closer the rate functions tend to reflect sensory magnitudes (Pfaffmann, 1960). Guttman's (1954) important study of bar pressing on an intermittent reinforcement schedule showed that relative rates of
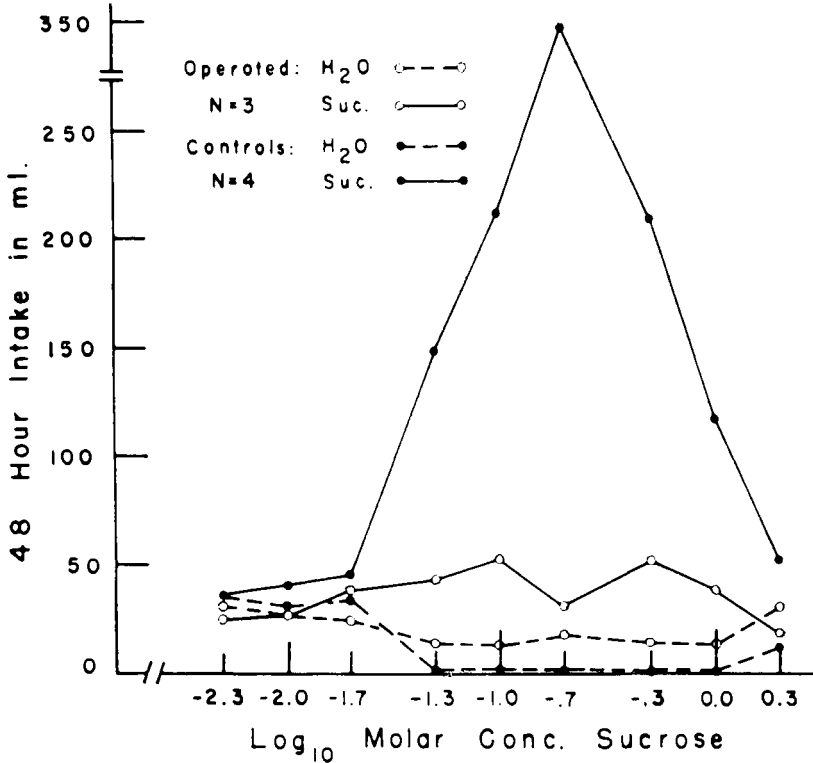
FIG. 9. Attenuation of two bottle preference for sugar following ablation of most of the thalamic gustatory relay.

response to glucose and sucrose were the same as their relative sweetness for man. The same relationship held for the relative taste effectiveness for the rat's chorda tympani response for these two sugars (Hagstrom and Pfaffmann, 1959).

Collier (1961) has worked out the detailed parameters of reinforcement in a bar pressing situation in terms of stimulus intensity, volume, and interval between reinforcements. He concludes that three independent sets of events control ingestive behavior : (a) the sensory properties of the substance being ingested, (b) the physical properties of the momentary post-ingestive load, perhaps a non-specific osmotic gastric factor, and (c) nutritive condition of the organism.

These same factors might be singled out in *all* studies of ingestive behavior, whether one-bottle or two-bottle, short term or long term, in need-free or deprived states, but the relative weighting of each of these factors and their interactions will differ in different situations. In the foregoing account, I have tried to enumerate how each of these physiological mechanisms control the various behaviors included under the general
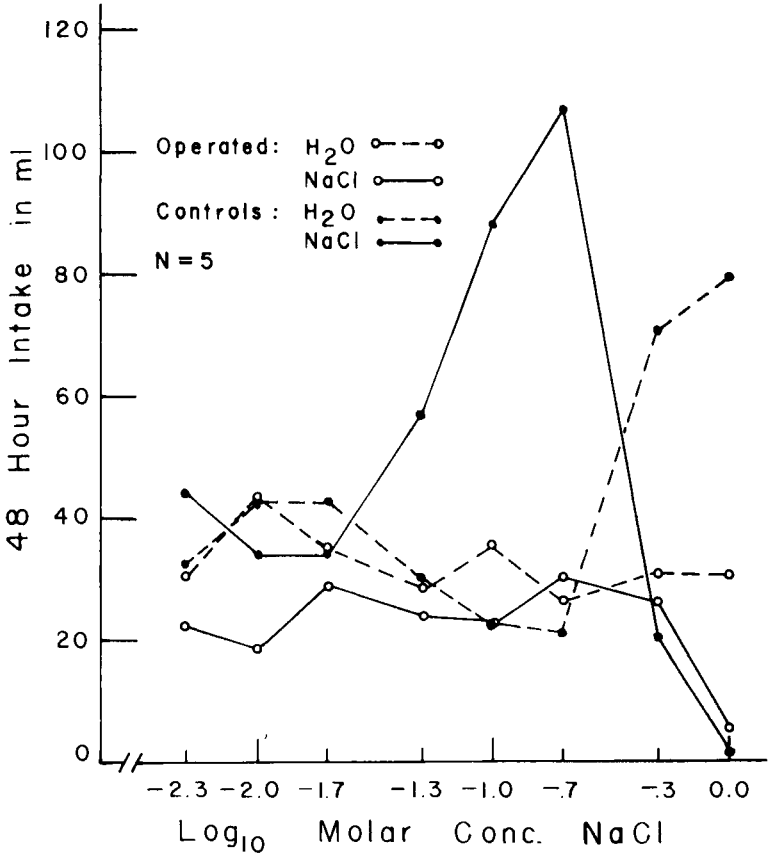
FIG. 10. Same as Fig. 9 for NaCl solutions.

rubric of taste preference. It is clear that we have come a long way from the situation where we expect to find simple relations between the physiology of taste and behavior. That there is a relation is clear, but it is often more complex than appears at first glance. Sophisticated behavioral analysis has made as important a contribution to our growing insight among these relations as have increasingly sophisticated physiological techniques.

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CHEMICAL CODING IN TASTE—TEMPORAL PATTERNS

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MUCH careful consideration has been given to the relationships between the chemical characteristics of gustatory stimuli and the magnitude of the neural response, i.e. the maximum or peak magnitude of the response to stimuli. There has been considerably less interest in the relationships between stimulus characteristics and changes in response magnitude over time.

The temporal characteristics of gustatory neural responses have been studied for only a few chemicals. Sodium chloride has received the most attention. A number of workers (Beidler, 1953, 1955, and 1961 ; Pfaffmann, 1955 ; Zotterman, 1956 ; Fishman, 1957 ; Halpern et al., 1961) have reported that the response to NaCl recorded from the rat chorda tympani shows a rapid rise to peak magnitude, a brief initial transient, and then a rapid fall to a relatively maintained response level. The latter is generally considered the adapted response level. In contrast, human psychophysical responses to NaCl have been reported to fall to or near zero quickly, sometimes within 10 sec (see : Pfaffmann, 1959, p. 37 ; Beidler, 1961, p. 124). Therefore, it has been proposed that gustatory responses in the CNS may be less well sustained than in peripheral nerves (Beidler, 1953, 1961). Before considering peripheral nerve responses in detail, let us briefly compare chorda tympani vs. CNS gustatory responses. Figure 1 is summated neural responses to stimulation of the anterior portion of the rat's tongue with 0.1 M NaCl. The records read from right to left. The lower record was made from the whole chorda tympani nerve. The multiunit neural activity was led off the nerve through silver–silver chloride wick electrodes. Here, and in subsequent figures, 25 ml of stimulating solution flowed through the tongue chamber and over the tongue in approximately 8 sec. The neural activity was passed through a Grass P–5 a.c. preamplifier, then led through a separator, which was set to remove much of the background noise, and then into a summator. The summator rise time was usually 0.75 sec. The fall time was always 7.5 sec. The summator output drove a critically damped Texas recti-riter. The upper

record was made with a 25μ nickle chrome electrode in the rostral medulla oblongata, presumably in the rostral pole of the nucleus of the fasciculus solitarius (see Pfaffmann *et al.*, 1961). The temporal characteristics of the chorda tympani and the bulbar responses are quite similar. Thus, one can find, at this level of the CNS, well sustained responses to 0.1 M NaCl.
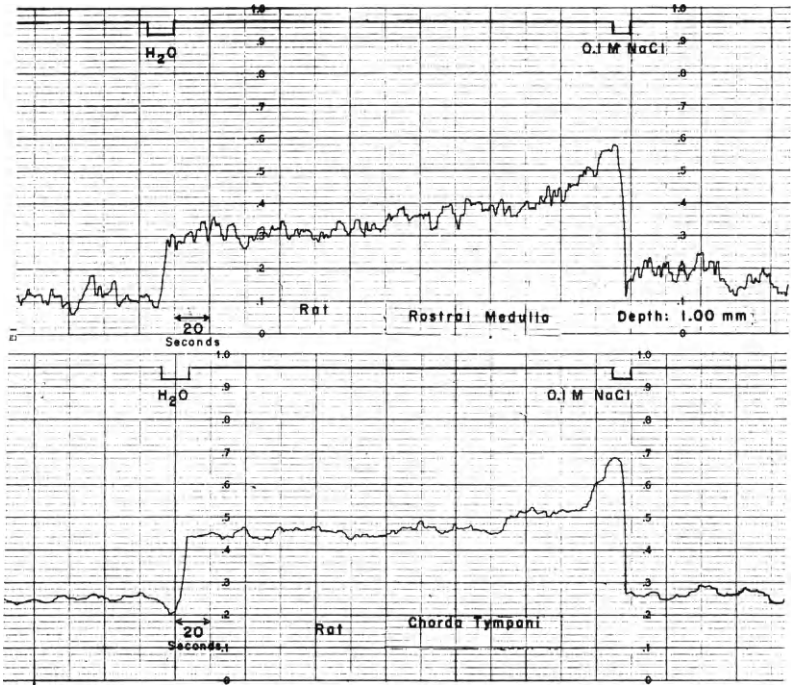

FIG. 1. Summated neural responses to chemical stimulation of the anterior portion of the tongue of the rat. *Upper record* : Response recorded with 25μ enamelled nickle chrome electrode 1.00mm ventral to the dorsal surface of the medulla, 1.9mm lateral to the median dorsal sulcus, and 2.7mm rostral to the obex. *Lower record* : Response recorded from the entire chorda tympani nerve of another rat. In both cases, downward deflection of the signal lines at the top of the records indicates the duration of chemical flow through the flow system and closed tongue chamber. The signal lines have been retouched.
Records read from right to left.

We will now concentrate on peripheral nerve responses. Are the temporal patterns of gustatory responses modified by changes in concentration? Figure 2 compares summated rat chorda tympani responses to 0.1 M and 0.01 M NaCl. The figure reads from right to left. The response to 0.01 M NaCl, on the left of the figure, rapidly fell to a very small adapted level. The response decreased to 30 per cent of the peak magnitude within 10 sec. Four minutes after stimulus application, this adapted response to

0.01 M NaCl was 15 per cent of peak magnitude. In contrast, the response to 0.1 M NaCl, shown here on the right, was 78 per cent of peak magnitude 10 sec post-peak. Four minutes post peak, this response to 0.1 M NaCl was 58 per cent of peak magnitude.
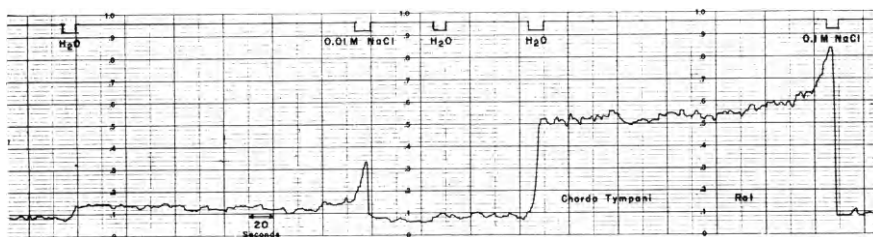

FIG. 2. Summated neural responses recorded from the entire chorda tympani nerve of one rat following chemical stimulation of the anterior portion of the tongue. The record is continuous, and reads from right to left. Downward deflection of the signal line, at the top of the record, indicates duration of chemical flow through the flow system and closed tongue chamber. The signal line has been retouched.

For the rats studied to date, the median response to 0.1 M NaCl at 20 sec post peak was 86 per cent of peak magnitude. After the same period of time, the median response to 0.01 M NaCl was 34 per cent of peak magnitude. About 4 min post peak, 0.1 M NaCl was down to 67 per cent, while 0.01 M had a median adapted level of 27 per cent of peak magnitude. Responses to 0.05 NaCl appeared to decrease faster than those to 0.1 M NaCl but slower than the responses to 0.01 M NaCl. Thus, under these recording conditions, rate and degree of adaptation of NaCl responses appear to decrease as stimulus concentration increases.

Recently, it has been observed that tongue stimulation with amino acids can produce responses in the rat chorda tympani with time courses different from the responses to NaCl (Halpern et al., 1961). The response magnitude produced by amino acids such as glycine and the stereoisomers of alanine are generally smaller than the rat chorda tympani response to 0.05 M NaCl (the response to 3.0 M glycine is between 0.05 M NaCl and 0.1 M NaCl) (Fig. 3A, B).

Oscillograph records indicate that responses to 1.5 M glycine and 1.2 M DL-alanine build up to peak magnitude slowly and do not have the initial large spike, high frequency burst found with responses to 0.1 M NaCl (Figs. 4, 5). In addition, responses to 1.5 M glycine and 1.2 M DL-alanine have post peak magnitudes much closer to peak magnitude itself than do responses to 0.1 M NaCl. Summator records (Figs. 6, 7, 8) likewise demonstrate slow rise times and well maintained responses for these amino acids. Time to peak magnitude appears to be a function of concentration, type of amino acid, and configuration (Fig. 9).

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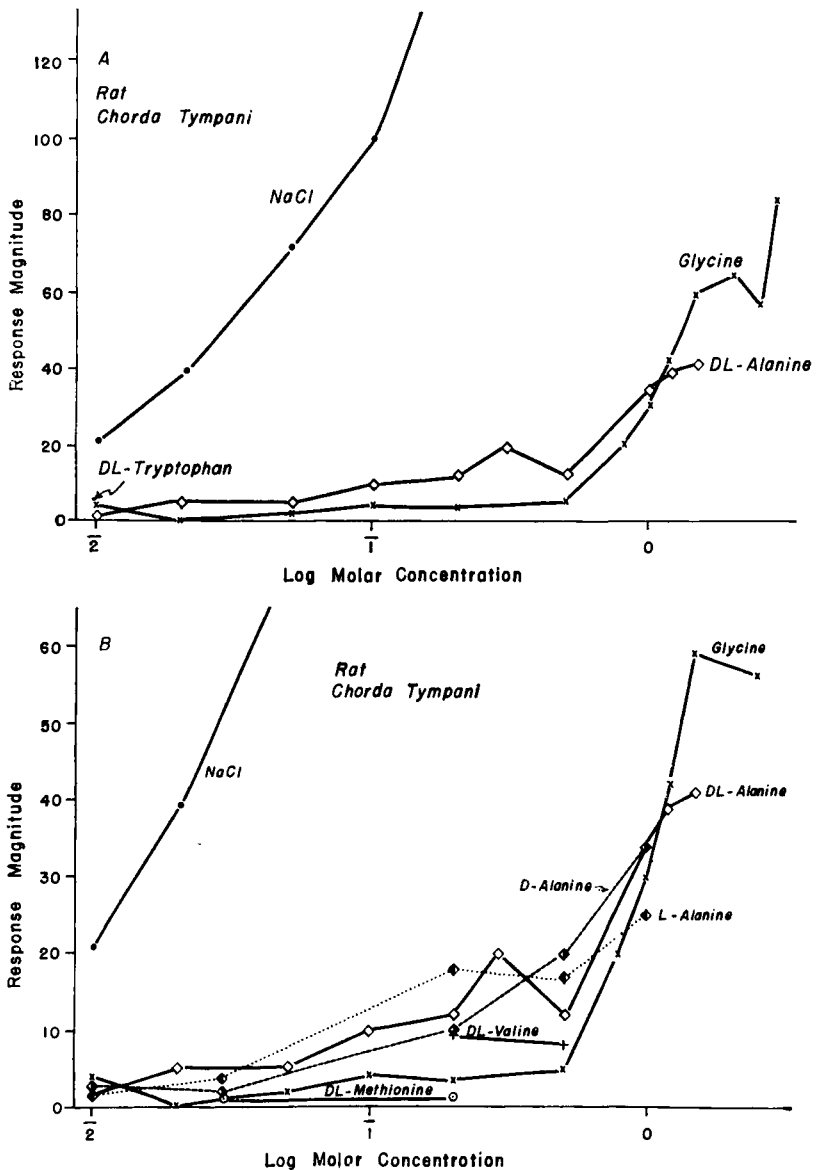
FIG. 3. Chorda tympani median summated response magnitudes following chemical stimulation of the anterior tongue of the rat (based on sixteen rats). Ordinate represents magnitude of the summated neural response in arbitrary units, adjusted to 100 units for the response to 0.1 M NaCl. A, responses to NaCl, glycine, DL-alanine, and DL-tryptophan, B, responses to NaCl, glycine (except 3.0 M) alanine (D-, L-, and DL-), DL-valine, and DL-methionine. Ordinate expanded (twice).

Reproduced from : Halpern, B. P., Bernard, R. A., and Kare, M. R. 1961. Amino acids as gustatory stimuli in the rat. *J. Gen. Physiol.* **45,** 681.

The foregoing data illustrate five characteristics of chorda tympani multiunit responses to relatively high concentrations of glycine and alanine: (1) Relatively long latency.   (2) Slow increase in response magnitude. (3) Little if any large spike, high frequency initial burst.   (4) Little adaptation after reaching peak magnitude.   (5) Stereoisomer specificity.

FIG. 4. Oscillograph records of electrical activity in the entire chorda tympani nerve of one rat following chemical stimulation of the anterior portion of the tongue. A flow system and closed tongue chamber were used. A stimulus onset signal was not used. The records have been aligned with the beginning of the response. In each case, flow duration was approximately 8 sec. Water wash always occurred after the end of the records. (A) H_2O (no obvious response), (B) 0.1 M NaCl, (C) 1.5 M Glycine, (D) Continuation of C, with 4.6 sec gap, (E) 0.1 M NaCl, (F) Continuation of E, with 4.6 sec gap, (G) 1.2 M DL-alanine, (H) Continuation of G, with 4.6 sec gap. (I) 0.01 M NaCl. The output of the summator preamplifier, which passes only positive pulses, was displayed on a Tektronix 502 oscilloscope and photographed with a Grass C4F camera. Records read from right to left.

Responses to 0.1 M NaCl have temporal characteristics directly opposite to the above pattern (characteristics one through four). This contrast between the time course of responses to NaCl vs. amino acids is somewhat similar to the comparison drawn by Pfaffmann (1955) between responses to

NaCl vs. sugars and to Beidler's (1953) comparison of responses to NaCl vs. $CaCl_2$ or $MgCl_2$.

The temporal characteristics of multiunit summated gustatory responses to stimulation of the anterior portion of the tongue of the rat with NaCl,
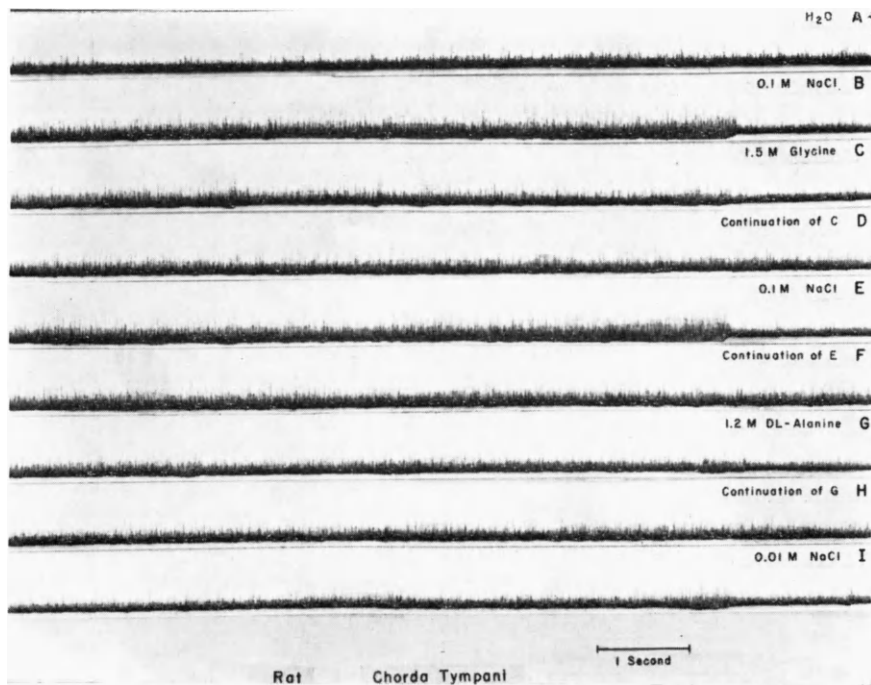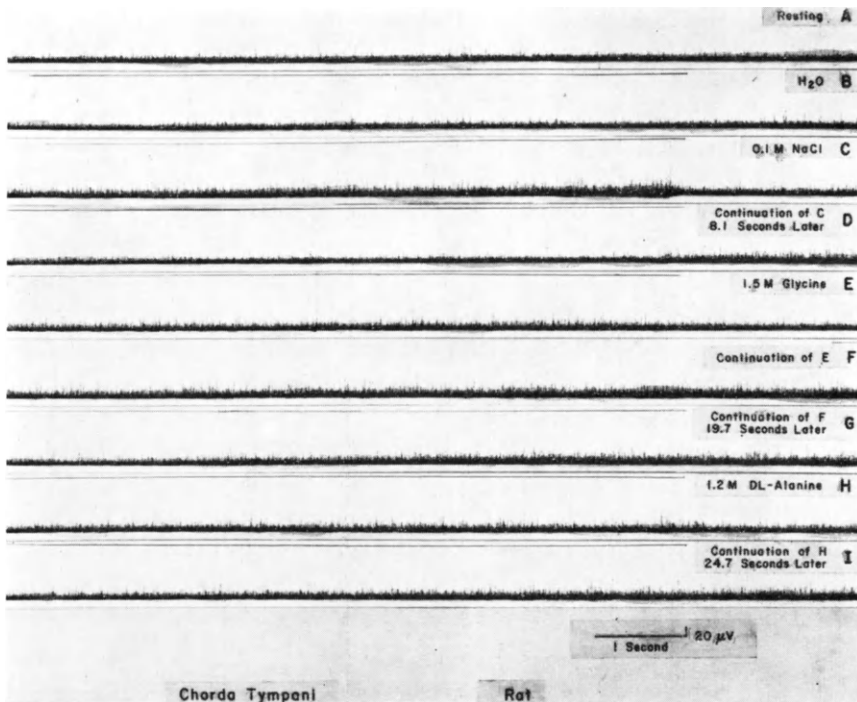

FIG. 5. Oscillograph records of electrical activity in the entire chorda tympani nerve of one rat following chemical stimulation of the anterior portion of the tongue. The signal lines at the tops of the records indicates the approximate duration of stimulus flow over the tongue. The signal lines have been retouched. (A) Resting. No liquid flow, (B) H_2O, (C) 0.1 M NaCl, (D) Continuation of C, 8.2 sec later. The signal line indicates a distilled water wash, (E) 1.5 M glycine, (F) Continuation of E, (G) Continuation of F, 19.7 sec later. The signal line indicates a distilled water wash. (H) 1.2 M DL-alanine, (I) Continuation of H, 24.7 sec later. The signal line indicates a distilled water wash. Stimulating and recording conditions as in Fig. 4. Records read from right to left.

glycine, and alanine have been considered. The temporal pattern of a response recorded from the chorda tympani varies with the concentration, the empirical, and the structural formula of the stimulating chemical. These data suggest that changes over time in the magnitude of gustatory responses may be of some importance in chemical coding in taste.
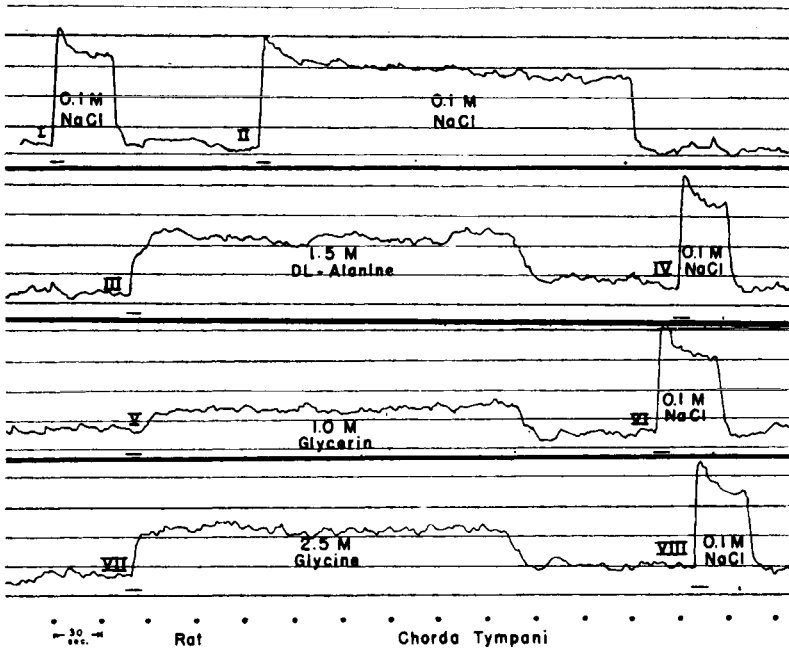
FIG. 6.   A tracing of summated neural responses recorded from the entire chorda tympani nerve of one rat following chemical stimulation of the anterior portion of the tongue.   The record is continuous, and is arranged from top to bottom of the figure.   The record reads from left to right.   The short horizontal lines close to the beginning of each response indicate the duration of stimulus flow through the tongue chamber.   The dot near the end of each response indicates the beginning of a distilled water wash.   The summator rise time was 0.5 sec.
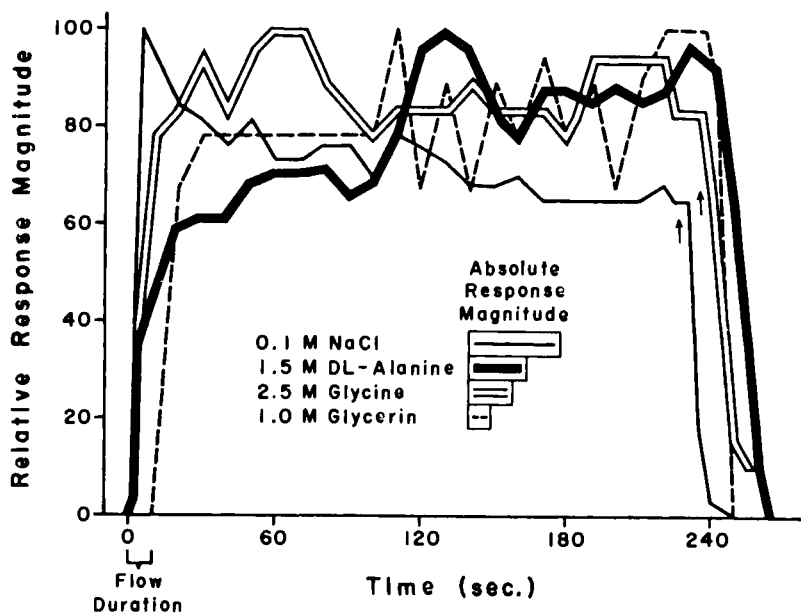
FIG. 7. Chorda tympani summated response magnitudes following chemical stimulation of the anterior tongue of one rat. Ordinate represents magnitude of the summated neural response to each chemical in arbitrary units, adjusted to 100 units for the maximum (peak) response for each chemical. The bar diagram represents the peak summated neural response magnitudes to the four chemicals, all on the same arbitrary scale (e.g. the response to NaCl was 41mm pen deflection). The tongue chamber is filled with stimulating fluid for approximately 10 sec. Summator rise time (RC) = 0.5 sec. Arrows indicate onset of H_2O rinse. Reproduced from Halpern, B. P., Bernard, R. A., and Kare, M. R. 1961. Amino acids as gustatory stimuli in the rat. *J. Gen. Physiol.* **45**, 681.

| Stimulus solution	Time to peak response, *sec.*	Response magnitude at selected time intervals after first reaching peak, expressed as a percentage of the peak response						
		2 sec	5 sec	20 sec	51 sec	67 sec	100 sec	230 sec
0.1 M NaCl	13	99	95	86	74	70	—	67
1.0 M glycerin	35	97	90	100	79	72	—	—
2.5 M glycine	56	100	97	86	100	86	—	—
1.5 M DL-alanine	118	97	90	84	85	100	90	—

FIG. 8. Temporal characteristics of the chorda tympani summated neural responses to one concentration of NaCl, glycerin, glycine, and DL-alanine

Table values are medians based on a total of eleven rats. Dashes indicate that no median data are avilable for that postpeak interval.
Reproduced from : Halpern, B. P., Bernard, R. A. and Kare, M. R. 1961. Amino acids as gustatory stimuli in the rat. *J. Gen. Physiol.* **45**, 681.
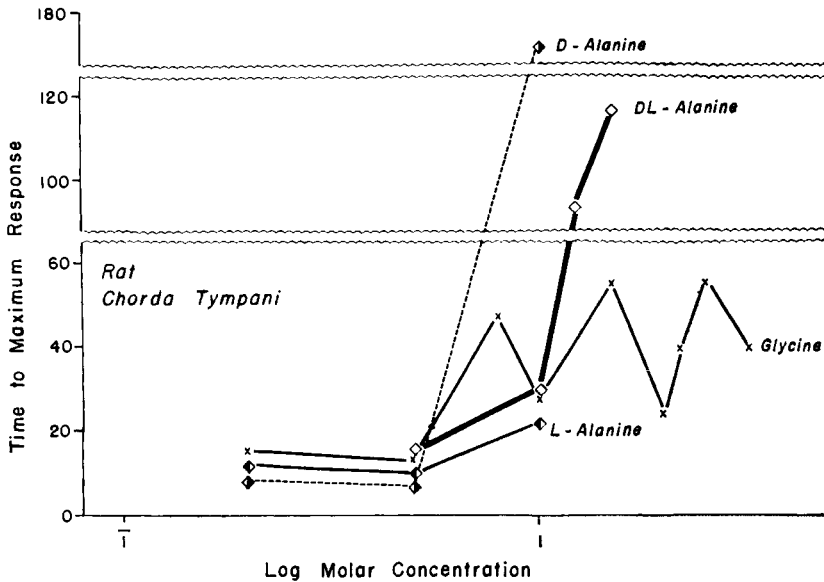
FIG. 9. Chorda tympani median time to maximum response magnitude following chemical stimulation of the anterior tongue of the rat (based on eleven rats). Ordinate represents time, in seconds from beginning of fluid movement in flow system (2 sec required to reach tongue, tongue chamber empty approximately 8 sec later) to development of maximum (peak) response magnitude. Summator rise time (RC), 0.75 sec.   Reproduced from : Halpern, B. P., Bernard, R. A. and Kare, M. R. 1961.   Amino acids as gustatory stimuli in the rat. *J. Gen. Physiol.* **45**, 681.

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COMPARATIVE STUDIES ON THE
SENSE OF TASTE

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How does the loss of the sense of taste affect an animal? Would the loss be more critical in one species than in another? There are no adequate answers to these questions because the function of taste in the physiological economy of the vertebrate animal body has not been established. We can only suggest possible roles for taste. The intricate relationship of taste, behavior diet and metabolism are, for the most part, still undefined. The objective of this paper is to present some comparative data on taste for species that differ in their natural diet, digestion and to some degree in their metabolism.

Although studies in taste have a long history (Boring, 1942), truly comparative studies have only been undertaken quite recently. Parker (1922) studying the chemical senses in a variety of invertebrates and fish was a pioneer in comparative studies. Behavioral responses of various domestic birds to taste stimuli were reviewed in a book by Engelmann (1957). Carpenter (1956) reported on a number of mammals and Kare (1962) described taste responses of various species of domestic animals. A comparative approach to electrophysiological studies has been used by Pfaffmann (1953), Beidler *et al.* (1955), Tamar (1956) and Zotterman (1961). The behavioral work with animals largely measures preference thresholds and the electrophysiological recordings are more likely to be related to discrimination thresholds. Therefore, it is difficult to correlate these two phases of taste research.

Comparative studies, when carried out with a sufficient number of species, can lead to generalizations about taste responses in animals. This may permit the separation of the unique from the fundamental. Of course, comparative research could be an end in itself, leading to an understanding of the evolution of taste.

The comparative approach in taste research has received limited use for several reasons. One was the rather prevalent belief of early sensory physiologists that the stimulus was all important and the kind of animal used was rather secondary. This led to the inference that all animals would react similarly to a given chemical. This concept has been demon-

strated to be erroneous using both electrophysiological and behavioral techniques. The emphasis on man in the very early studies was probably due to the ease of testing. The development of more objective testing procedures was an important prerequisite to using the lower animals. Perhaps the practical problem of maintaining large animals, or an extensive array of animal species, has limited comparative studies. Another factor delaying the appearance of comparative studies in taste was the rather belated infusion of evolutionary principles into physiological research. Comparative studies in anatomy had been popular for a long time before Darwin, but this was not as true in physiology. This may have been due to an apparent lack of applicability of physiological characters in taxonomy, with which many of the zoologists of the time were concerned.

One should consider function of taste in different animals, since it is on this aspect that natural selection is acting. Unlike the other chemical senses, which may have a variety of functions (i.e., mate selection, avoidance of predators), the function of taste is more limited. It is reasonable to ascribe to it a role in the regulation of ingestion of nutrients and possibly the avoidance of toxic substances. It would be expected that the taste system in a particular species would be adapted through the evolutionary process to its metabolic and dietary requirements.

The diet of animals in the wild is characteristic of the species. Although chromatographic and other techniques may eventually make it possible to work with the complex taste mixture of natural diets, sound experimental design limits us to reagent grade chemicals. Only with such chemicals can one confidently compare results or repeat experiments.

A range of concentrations is used since species vary in degree of sensitivity. In addition, a series of related chemicals are tested since this could reveal a pattern of taste behavior which might be similar, yet not identical.

Using the above criterion, Kare and Medway (1959) measured the reaction of the fowl to a series of sugars in a two-choice situation where distilled water was the alternative. The sugars were selected with a view to introducing variables such as osmotic pressure, viscosity, nutritive value, melting point, configuration, toxicity, solubility, rotation, sweetness, and so forth. Some of the results are summarized in Fig. 1. Parenthetically, I would interject that the experiment failed to suggest any chemical or physical basis to explain the fowl's preference reactions.

The reaction of calves to common sugars is illustrated in Fig. 2. Even a superficial examination leaves no doubt that the differences in reaction between these two species are more than one of degree. Further, there is no common pattern. This is amply illustrated with xylose which the fowl finds markedly unacceptable and the calf obviously relishes.

In Fig. 3 the response of rats to a variety of sugars was measured. The range of concentrations tested was more extensive than with the fowl. It

is obvious that the rat is different from the fowl in these responses. Careful observation also reveals fundamental differences from the calf. A good example of this is the reaction to maltose. The calf was apparently indifferent to this sugar at concentrations up to 4 per cent while it was the most preferred sugar by the rat.

SUGAR PREFERENCES / WATER (CHICKS)
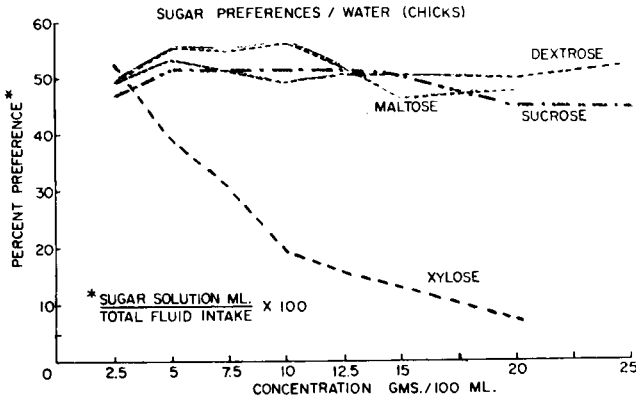
FIG. 1. The response of the chick to various sugar solutions in a two-choice preference test is illustrated. The response to dextrose, sucrose and maltose at all concentrations was one of indifference, i.e., close to 50 per cent. Xylose was actively rejected.

SUGAR PREFERENCES / WATER (CALVES)
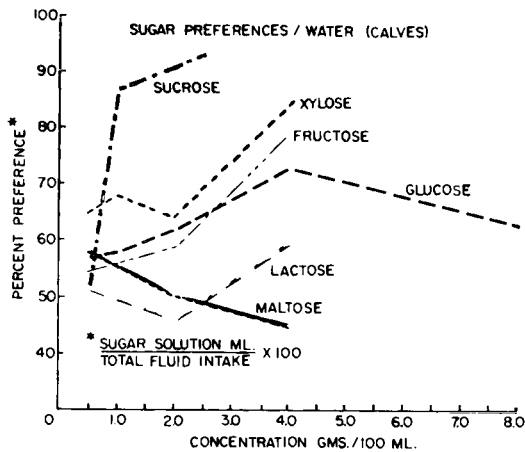
FIG. 2. The response of the calf to various sugar solutions in a two-choice preference test is illustrated. With the exception of maltose and lactose marked preference for the sugars are indicated.

These animals are not closely related phylogenetically and the physiological differences between them can be exemplified by the diversity in digestive systems with correlated differences in diet.

In the dog, a carnivore, data on a wide range of concentrations have not

been completed ; however, the response to dextrose, sucrose, maltose and fructose suggests yet another pattern of response to the sugars.

On the other hand, preference thresholds for some sugars in insects are similar to those of the vertebrates (Prosser and Brown, 1961.) Perhaps this convergence is related to similar selective pressures. Certainly substances containing natural sugars are eaten by both insects and vertebrates, and consequently natural selection could have favored responses to similar concentrations in the two groups.

SUGAR PREFERENCES / WATER (RATS)
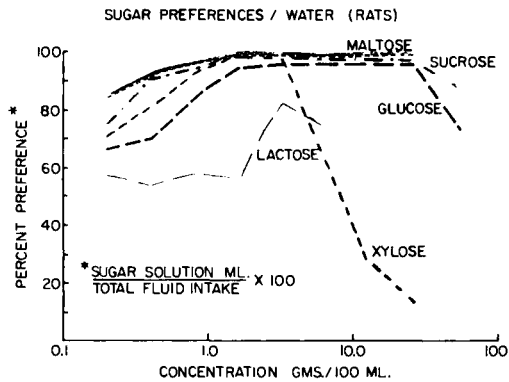
FIG. 3. The response of the rat to various sugar solutions in a two-choice preference test is illustrated.

In an early paper on chickens (Kare *et al.*, 1957) a slight preference for sucrose was reported. Subsequently, in a more extensive study on sugars (Kare and Medway, 1959), as was illustrated in Fig. 2, the chick was found to be totally indifferent to sucrose and glucose solutions.* This contradicted the marked preference for sugars by chicks reported by Jacobs and Scott (1957). Assuming all the results were accurate, there were apparently uncontrolled variables.

The effect of a caloric deficiency on sugar preference in the chicken is illustrated in Fig. 4 (Kare and Halpern, 1962). When fed, ad libitum, the chick exhibited no preference for a 10 per cent sucrose solution where distilled water was the alternative. However, where feed was limited to 75 per cent of that consumed by the controls, a preference for sucrose solution became marked and in addition fluid intake almost doubled. Evidently, the caloric needs of the animal lead to adaptive behavior. The figure also indicates that animals which were initially on a restricted diet continue at least for a time to show the same behavior, i.e., preference for sucrose, after being placed on an adequate diet. It follows that it is possible for a demonstrated preference for sugar to be related to a prior

*This was in accordance with the results obtained by electrophysiological methods (Kitchell *et al.*, 1959).

dietary deficiency. It seems reasonable that differences in adequacy of diets could explain discrepancies in the reports on preference behavior of chicks for sucrose.

While cats have been frequently described as being indifferent to sucrose, Frings (1951) reported contrary results. He found that cats do distinguish between diluted milk with sucrose and that without, when starved 24 hr.
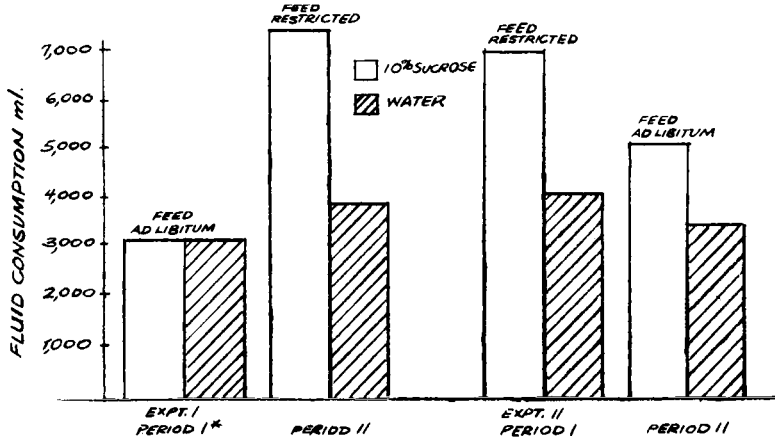
FIG. 4. The influence of nutritional state on preference and intake of sucrose in solution
*Periods represent successive 18 day trials.

Although this study with chicks was designed to measure the influence of caloric deficiency on preference behavior, other nutritional deficiencies or imbalances (e.g., LeMagnen, 1956) could modify preference behavior. Bernard *et al.* (1961) reported that vitamin A depleted rats show abnormal taste behavior. An inherent " nutritional wisdom " of domestic animals cannot be relied on in preference trials. In recent studies in our laboratory with protein deficient chicks they failed to prefer a casein solution in a two-choice situation.

The differences in response to sugars by the chick, the rat and the calf might also be considered with relation to their circulating blood glucose level and their physiological regulating mechanisms. The chicken normally has a blood glucose level that would be described as diabetic for the rat, while the cow normally has a low blood sugar level that would be in a pathological category for the rat. Further, both the chicken and the cow are refractory to insulin, the blood sugar regulating hormone.

Expressing preference as per cent of fluid intake gives only part of the picture for sugars. Xylose is preferred by the rat to about the same degree as glucose, maltose and sucrose (i.e., 97 per cent or over, Fig. 5). However, while the common sugar solutions cause a doubling of fluid intake, the volume of xylose solution consumed remains close to the control. It

might be suggested that this is related to nutritive value. However, this is unlikely since a saccharin solution is preferred equally as well as xylose and fluid intake is increased as for the other sugars. The restricted fluid intake
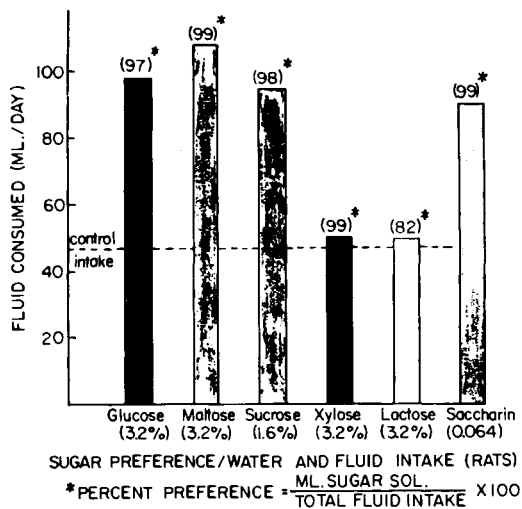
FIG. 5. The preference for sugar solutions and the influence on total fluid intake is illustrated. While xylose is preferred by the rat to about the same degree as glucose, maltose or sucrose, it fails to increase fluid intake in a similar manner.
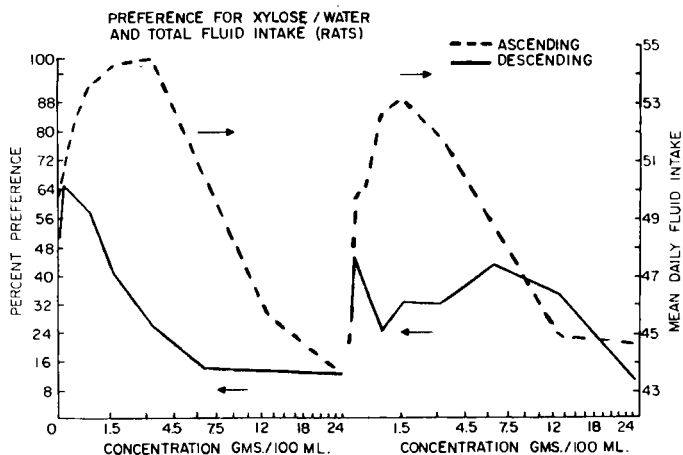
FIG. 6. The effect of prior ascending or descending concentration sequences on subsequent preferences on the left and on fluid intake on the right (xylose).

could be related to the toxicity of xylose. However, this theory requires additional support. The results with lactose are interesting since preference is less than that encountered with the common sugars while total fluid intake is close to that of the control.

Prior exposure to sugars, particularly the high concentrations, had a pronounced effect on both preference and fluid intake. Figure 6 illustrates this divergence between ascending and descending sequences with xylose, and Fig. 7 presents similar data for lactose. The difference in response in the case of these two sugars is worthy of special note. The other sugars, i.e., glucose and maltose, have yet different shaped curves (Fig 8). A
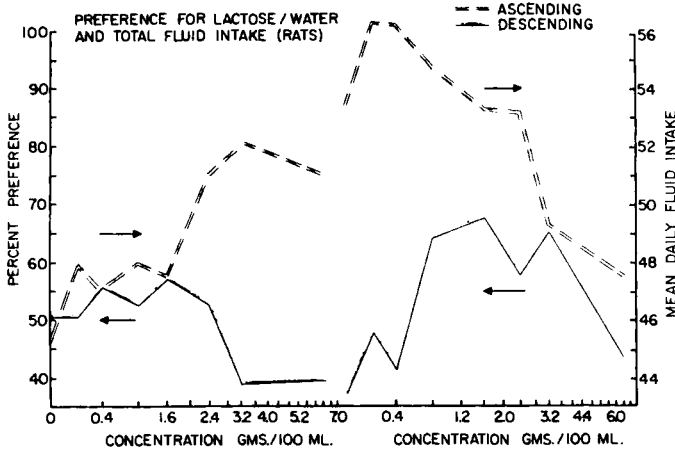
FIG. 7. The effect of prior ascending or descending concentration sequences on subsequent preferences on the left and on fluid intake on the right (lactose).
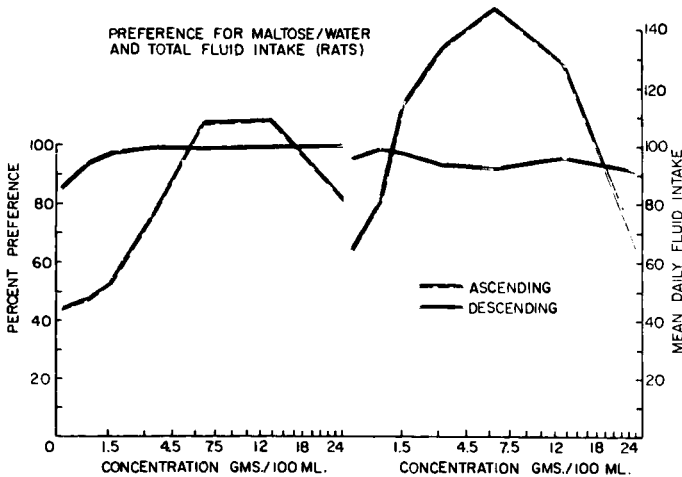
FIG. 8. The effect of prior ascending or descending concentration sequences on subsequent preferences on the left and on fluid intake on the right (maltose).

conservative observation would be that both the specific character of the sugar and the concentration sequence will influence the preference behavior

and volume consumed. The rate at which the changing concentration of the stimulus affects preference behavior appears to be a function specific for each chemical. Further there is no predictable relationship between the apparent hedonism and volume consumption.

Leaving the realm of metabolism, let us consider response to the non-nutritive saccharin. Figure 9 illustrates the range in response to this compound. On the basis of this work one can suggest that pigs and rats over a wide range of concentrations found it appealing while calves were indifferent. I might add that at a single concentration tested most chickens found it slightly offensive and dogs markedly so.
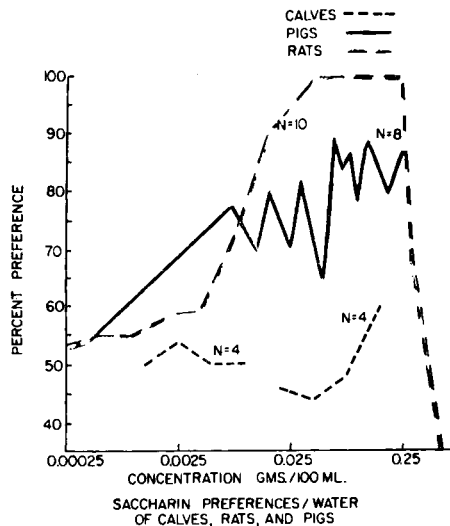
FIG. 9. A comparison of the response of calves, rats and pigs to saccharin solution.

On this point I should qualify the remark " most animals ". We found a minority of pigs that were offended (Kare, 1961) by saccharin at every concentration offered. Similarly most dogs actively rejected saccharin in low concentrations in their food, but a small minority tolerated if not preferred it.

This introduces the question of individual differences. Individual variation in taste ability (Ficken and Kare, 1960) was studied using 28 male and 28 female Barred Plymouth Rock-Rhode Island Red crosses, aged two months. A single stimulus method was employed (modified from Duncan, 1960) and the test substances were ammonium, calcium and ferric chlorides at three or four different concentrations.

Different individuals had markedly different thresholds (the lowest concentration at which the intake differed from that of water). For

example, in the case of calcium chloride, 17 apparently discriminated at concentrations of 0.2 per cent or lower, 12 at 0.8 per cent, 14 at 1.2 per cent, and 13 had thresholds higher than 1.2 per cent. Thus the distribution of thresholds was of a continuous type. There were no apparent sexual differences in taste ability. No individual was completely taste " blind " since all birds responded if the concentration reached a high enough level. A chemical specificity was involved, since an individual that could taste one chloride at a low concentration did not necessarily respond to other chlorides at low concentrations. A bird that was indifferent to one chloride often showed good taste sensitivity for another.

In addition to these marked individual differences in threshold, there were individual differences in preference behavior at low concentrations, i.e., some individuals preferring a chloride and others rejecting it. However, most birds showed a pattern of increasing rejection with increasing concentration.

Williamson (Pers. Comm.) has carried the study of individual variation to three generations. He screened 100 males for ferric chloride thresholds and selected two birds, the ones with the lowest and highest thresholds, and of 100 females selected 11 high and 9 low threshold birds. He then performed a selective breeding program, breeding the high threshold male with various high threshold females, and doing the same for low threshold birds. The progeny were then tested for ferric chloride thresholds at 8 weeks, the same age as the parents had been tested, to avoid introducing age as a variable. There was a statistically significant difference between those with high and those with low threshold parents, indicating that ferric chloride thresholds in the fowl have a genetic basis.

In other related studies we used 12 breeds of chickens and found some evidence of breed differences. Here again, it was one of degree rather than absolute difference. Nachman (1959) has shown in rats that certain individual differences in response to saccharin have a genetic basis.

Since in the experiments with sugar chickens had failed to respond except to xylose, work with series of sodium salts and various chlorides was undertaken (Tables 1 and 2). Again as with the sugars, no physical or chemical basis for preference (i.e., rejection) could be discerned. Toxicity was considered as a factor influencing preference since the chicks did reject some toxic salts. However, since some salts destroyed all the chicks, toxicity could not be consistently a variable. In the case of sodium tungstate at the higher concentrations intake constituted a lethal quantity while at the lower concentrations, although the salt was accepted indifferently, the threshold for toxicity is not reached.

One area in taste research that has been most interesting is the water response observed in some animals (Zotterman, 1961). Special concern was directed in our laboratory to the pH of the water used. The body

TABLE 1. THE PREFERENCE FOR SOLUTIONS OF SODIUM
SALTS AT VARIOUS CONCENTRATION ARE PRESENTED
PREFERENCE*/WATER (CHICKS)

	Concentration (gms./100 ml)				
	0.1	0.2	0.4	0.8	1.0
Na. Acetate	55	52	56	52	51
Na. Sulfate	54	52	52	53	50
Na. Phosphate (monobasic)	52	53	52	52	54
Na. Succinate	49	52	54	50	56
Na. Citrate	54	52	54	47	35
Na. Phosphate (dibasic)	51	49	47	44	14
Na. Tungstate	50	46	48	—	—
Na. Bicarbonate	52	43	38	20	14
Na. Benzoate	49	41	23	15	10
Na. Bisulfate	38	23	35	17	23
Na. Pyrophosphate	46	37	20	3	4
Na. Perborate	42	29	10	9	6
Na Carbonate	42	30	10	4	2
Na. Phosphate (tribasic)	46	20	4	1	2
Na. Cholate	4	20	3	—	3

$$*preference = \frac{salt\ solution\ consumed \times 100}{total\ fluid\ intake}$$

TABLE 2. THE PREFERENCE FOR CHLORIDE SOLUTIONS
OVER DISTILLED WATER AT VARIOUS CONCENTRATIONS ARE
PRESENTED.

PREFERENCE/WATER (CHICKS)

	Concentration (gms/100 ml)				
	0.1	0.2	0.4	0.8	1.0
Sodium Cl.	50*	50	55	50	45
Magnesium Cl.	49	51	51	53	45
Choline Cl.	51	48	49	50	51
Manganese Cl.	49	51	46	28	16
Strontium Cl.	50	38	44	18	9
Ammonium Cl.	49	46	35	12	6
Barium Cl.	36	48	41	—	15
Calcium Cl.	43	45	27	15	5
Zinc Cl.	33	24	10	2	2
Cobalt Cl.	26	12	6	5	6
Tin Cl.	30	7	1	1	2
Copper Cl.	6	11	3	8	4
Iron Cl. '	2	4	2	3	4
Cadmium Cl.	———————— lethal ————————				
Lithium Cl.	———————— lethal ————————				

regulation of pH is perhaps one of the body's most sensitive mechanisms. It was reasonable to consider that the water response was in part a sensitivity to pH. For this reason we tested the reaction of chicks (Fuerst and Kare, 1962) and also calves to pH (Kare). In Fig. 10 is a summary of the results with chicks. The most startling observation is the tolerance, taste-wise, for acid. The results with calves are similar, although not as dramatic. Thus the pH of the water used in electrophysiological studies is probably not an important factor.

Percent intake of acids and bases at different pH levels

pH	1.0		2.0		3.0		4.0 —
Acids							
HCL	4	19	50		59		
H₂SO₄	15	35	54		56		
HNO₃	8		62		52		
Acetic					16		53
Lactic				15	61		

pH	— 10.0		11.0		12.0		13.0
Bases							
NaOH		45	47			33	2
KOH			48		36		3

FIG. 10. Tabled values are the mean of replicate lots. The per cent intake

$$= \frac{\text{volume of tested fluid}}{\text{total fluid intake}} \times 100 \text{ (18 daily values were averaged). The}$$

position of the numbers is an indication of the pH of the test solution. For example at pH 1.5 the average daily consumption of HCl was 19 per cent of the total fluid intake. Distilled water was the alternative in every instance.

The question still remains, what is the function of taste in animals? The mean number of taste buds in a variety of species are presented in Table 3. We know that the cow with 25,000 taste buds does not respond

TABLE 3. THE MEAN NUMBER OF TASTE BUDS DESCRIBED FOR DIFFERENT ANIMALS ARE PRESENTED.

Chicken	24	Lindenmaier and Kare, 1957
Pigeon	37	Moore and Elliott, 1946
Bullfinch	46	Duncan, 1960
Starling	200	Bath, 1906
Duck	200	Bath, 1906
Parrot	350	Bath, 1906
Snake	0	Payne, 1945
Kitten	473	Elliott, 1937
Bat	800	Moncrieff, 1951
Human	9,000	Cole, 1941
Pig and Goat	15,000	Moncrieff, 1951
Rabbit	17,000	Moncrieff, 1951
Calf	25,000	Weber, Davies and Kare, 1961
Catfish	100,000	Hymen, 1942

behaviorally to chemicals that evoke strong rejection in the fowl who has only a few dozen. What if any is the correlation between gross anatomy of the receptors and taste behavior? This numerical enigma parallels many others in taste which so far affects no pattern of function.

The collective preference results presented here indicated that the four qualities of taste used in human work serve no purpose with animal behavior. On the contrary, the results with sugars support a concept of separate taste worlds for each species.

No pattern, chemical, physical, nutritional or physiological, can be offered to explain the collective comparative results. However, the apparent contradictions and exceptions in response behavior do not deny the existence of an overall theory.

Man, monkeys and the guinea pig, amongst the animals tested, are known to require vitamin C, rats are uniquely independent of nicotinic acid, only chickens require the simple amino acid glycine, the Dalmatian dog fails to use uricase in protein metabolism ; pigs have their unsaturated fatty acid on the outside of the glyceride while other animals have the unsaturated in the middle. It follows that a species uniqueness in taste response would have many precedents in nutrition and metabolism.

One cannot freely ascribe to animals the taste sensations experienced by man for specific chemical entities. The differences between species may be fundamental. They could challenge the widely held concept which infers a universal character to taste physiology more extensive than that of the endocrines.

The results presented here support a concept of differences in degree between individuals and absolute differences between species. Further, they question generalizations on taste, particularly those based upon human experience.

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THE VARIATION IN TASTE THRESHOLDS OF RUMINANTS ASSOCIATED WITH SODIUM DEPLETION

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AT a recent symposium on the physiological and behavioural aspects of taste, Tepperman (1961) discussed the inter-relationships of taste, metabolism and nutrition. He paid due tribute to the pioneer studies in this field of Richter who used the rat as the subject for experimentation (Richter, 1943). Examples of self-regulatory processes are numerous in certain deficiency states of ruminants, for example, Theiler, Green and Du Toit (1924) showed that phosphorus deficient cattle have a preference for bones (osteophagia). I should like to discuss a little further the relationship between taste and biochemical status in ruminant herbivores, with particular reference to sodium depletion.

Herbivorous animals generally, and ruminants in particular, often exist on a quite precarious mineral balance. This is because the vegetative diets contain comparatively little sodium and a great deal of potassium. The Na/K ratio of vegetable material often widens through the seasons and more especially if potassium salts are included in chemical manures (Dobson, Kay and McDonald, 1960). The ingestion of food relatively deficient in sodium is reflected in the sodium plasma level which may be the factor which produces the " drive " which causes wild ruminants to search vast areas for salt licks (Russell and Duncan, 1956). I believe that in Northern Sweden, the reindeer brought down to coastal regions appear to relish long draughts of seawater.

Firstly I should like to mention the results of experiments conducted to ascertain the preference for sodium bicarbonate on a number of housed goats. The care and management of the animals used in these experiments and the technique of the two-choice preference test adopted are identical with the description published earlier (Bell, 1959). Briefly, fluid was provided from two identical containers (3.7 l. bottles), each fluid container having more than sufficient fluid to satisfy the animal for 24 hr. For the test, one bottle was filled with a 1 per cent sodium bicarbonate solution in tap water and the other with tap water. The amount of the two liquids

remaining, tap water and sodium bicarbonate solution, was measured at the same time each day and the amount consumed noted. In order to prevent bias due to visual or behavioural cues the position of the test solution was reversed at the end of 24 hr. The amount of sodium bicarbonate solution taken during a 48 hr period was expressed as a percentage of the total fluid intake (i.e. test solution + water). These figures when plotted as the ordinate show graphically the amount of liquid ingested daily as sodium bicarbonate solution.

The goats had been housed indoors for some months prior to the experiments but they received an adequate diet including a balanced mineral mixture which was always available to be taken *ad lib.* In two of the animals sodium bicarbonate was introduced directly into the rumen compartment of the stomach by way of a previously prepared rumenal fistula. Coincident with the beginning of this treatment there was a marked reversal of the preference shown by the animals for the 1 per cent sodium bicarbonate offered as an alternative choice to tap water. In the animal receiving a daily supplement of 25 g the amount of sodium bicarbonate taken fell below 25 per cent of the total fluid intake and even further, to less than 10 per cent, in the animal receiving 50 g/day sodium bicarbonate supplement (Fig. 1).

These results suggest that the parenteral introduction of the sodium bicarbonate reduced the preference of the goats for sodium bicarbonate over tap water. It is unlikely that the behavioural change is due to alimentary osmotic effects since the total volume of liquid consumed remained at a steady level. It would appear rather that by increasing the sodium content of the plasma the sodium taste threshold had been lowered. The failure to return towards a sodium bicarbonate preference on discontinuation of the supplement suggests a repletion of sodium stores. There were, however, two paradoxical features of this experiment, firstly plasma Na and K levels showed little deviation from normal levels during the course of the experiment, and secondly the animals did not react to maintain their apparent sodium deficiency by taking sufficient quantities of the sodium salts available in the mineral lick.

A second experiment was set up designed to show the effect of smaller amounts of sodium salts using a similar arrangement and the goats again showed marked preference for 1 per cent sodium bicarbonate over tap-water.

After preference values had been established, a supplement of sodium bicarbonate was administered daily for a limited period through a stomach tube directly into the reticulo-rumen : by this means any permanent stress due to the presence of the rumenal fistula was obviated but the buccal gustatory receptors were not activated directly by the sodium bicarbonate supplement. The experimental animals showed a change in preference
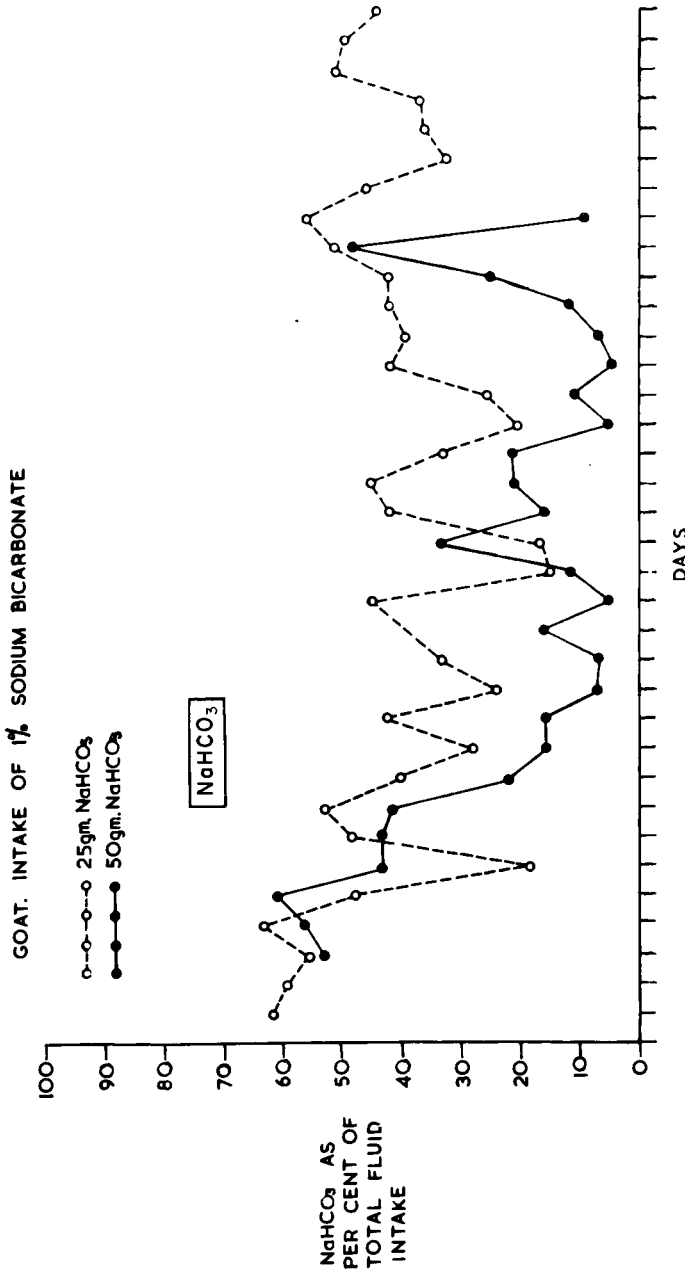
FIG. 1. Two choice preference test of 1 per cent sodium bicarbonate and water in goats. In the days covered by the black bar a supplement of sodium bicarbonate was administered directly into the rumen by way of a permanent rumenal fistula.
(From Bell, F. R., 1962. *J. Comp. Physiol. Psychol.*)
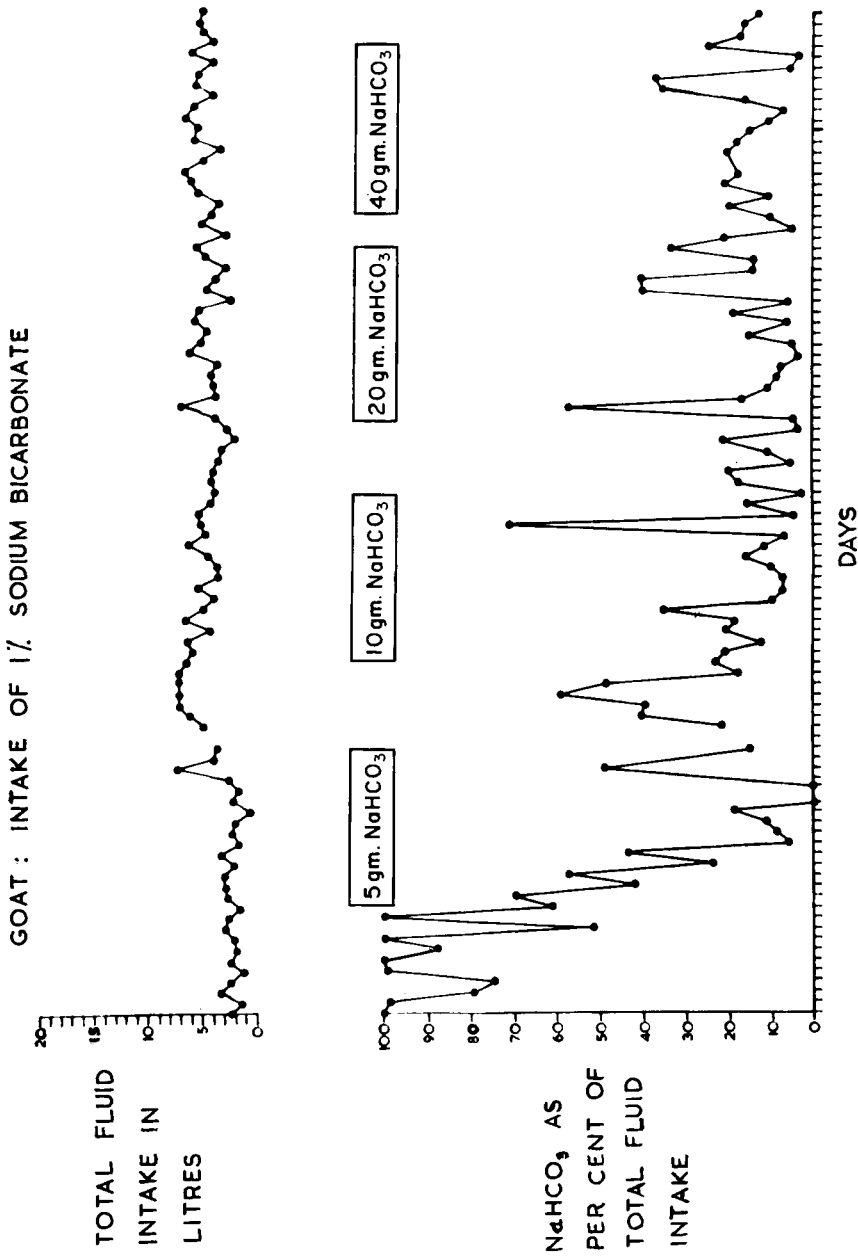
FIG. 2A. Two-choice preference test of 1 per cent sodium bicarbonate and water in goats. The amount of sodium bicarbonate supplement given per day is shown on the bar which covers the days when supplement was administered. The upper curve shows daily total fluid intake. (From Bell, F. R., 1962. *J. Comp. Physiol. Psychol.*)
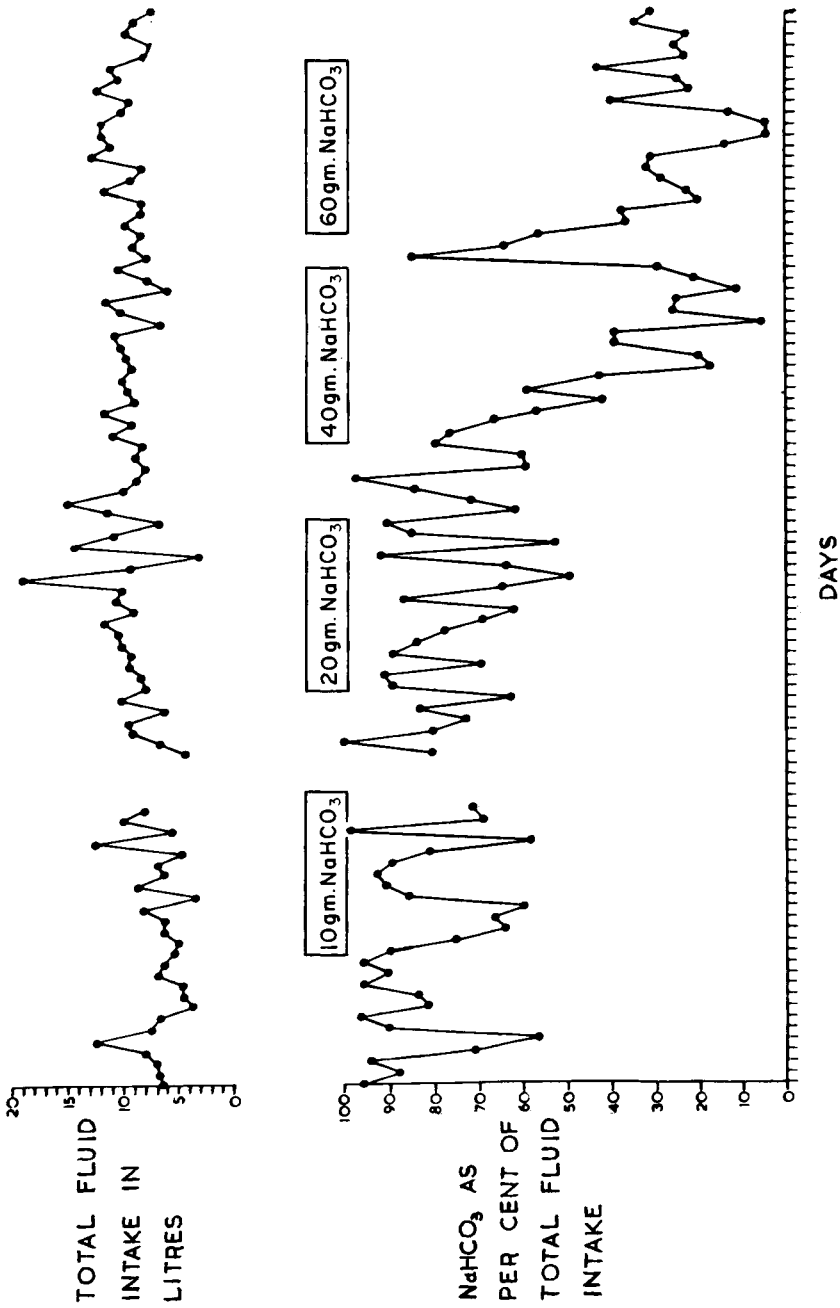
FIG. 2B

levels for the sodium bicarbonate solution almost immediately but with considerable variation between individuals (Fig. 2). In one animal a 5 g daily (Fig. 2A) supplement was sufficient to change an almost 100 per cent preference for sodium bicarbonate to a 100 per cent aversion whereas in another animal a daily supplement of 60 g/day was needed to reduce the animal's preference for sodium bicarbonate solution (Fig. 2B). In other goats the response was intermediate so that relatively small amounts of sodium bicarbonate altered the preference of the animals for sodium bicarbonate with the result that less alkali was ingested.

This demonstration that a preference for alkaline solution can be changed to an aversion by administering a supplement of sodium bicarbonate is striking. It is unlikely that the variation in taste is due to changes in sensitivity of the peripheral receptors for Pfaffman and Bare (1950) have shown in rats that salt deficiency following adrenalectomy does not alter the thresholds of sensitivity.

Apparently in the goats used in these experiments ingestion of sodium salt is being controlled by some process which is dependent upon the bodily supplies of sodium. It is possible that the controlling mechanism is mediated through the adrenal cortex either directly by variation in blood sodium levels or indirectly hormonally following the activation of metabolic centres of the hypothalamus or in some other part of the brain. It would appear that in the goats of these experiments the sense of taste is being used to regulate sodium levels in the body and provides a good example of the whole body self regulatory process postulated by Richter (1943).

The next experiments I want to discuss are concerned with variation in preference thresholds for sodium in monovular twin calves. Monozygous cattle twins were used because they have been shown to have very similar threshold values for taste (Bell and Williams, 1959). Denton (1957) has described a method of inducing sodium deficiency in sheep by exteriorization of the parotid duct when the continuous secretion of saliva causes a loss of sodium from the body. Sheep made sodium deficient exhibit an avidity for rock salt; the intake being 0.5–2.0 g/day in the normal state and 5–15 g/day when Na-depleted. The tendency towards sodium depletion in a sheep with a fistulated parotid duct can be balanced by feeding a daily supplement of 50 g $NaHCO_3$.

When the parotid duct of a calf is exteriorized the effects are very similar to those described for sheep. The salivary Na : K ratio is reversed from 145 m-equiv/l. : 4.5 m-equiv/l. to 5.4 m-equiv/l.: 132m-equiv/l., but it can readily be restored by feeding a supplement of NaCl or $NaHCO_3$. Salivary output from the exteriorized parotid duct decreases directly with the degree of sodium depletion. At the same time the animal shows aphagia, at first for concentrated food but later for all food including hay. The

degree of anorexia can be controlled by manipulating the salivary sodium level by giving or withholding sodium supplement. It would appear that the fall in the sodium level of the extracellular fluid parallels some mechanism which is controlling food intake. It should be remembered in this regard that the salivary sodium bicarbonate plays an essential role in the digestive process of ruminants. When an experimental calf is allowed continuous access to either 1 per cent NaCl or 2 per cent $NaHCO_3$ it will take drink sufficient to maintain its salivary Na/K ratio at normal levels whereas its control twin will take only minimal quantities.

After a period of depletion the taste thresholds for both members of the twin pair have been examined by the two choice preference technique (Bell and Williams, 1959). The experimental calf shows a much higher taste threshold for sodium chloride for it will take 5 per cent NaCl solution compared to the normal calf which will accept only 0.03 NaCl. Similarly in tests for $NaHCO_3$ taste thresholds the sodium depleted calf showed a higher acceptance threshold than its normal twin (Fig. 3). During the discrimination tests the salivary Na/K ratio is restored to normal in the experimental calf when it is imbibing the higher concentrations of either NaCl or $NaHCO_3$.
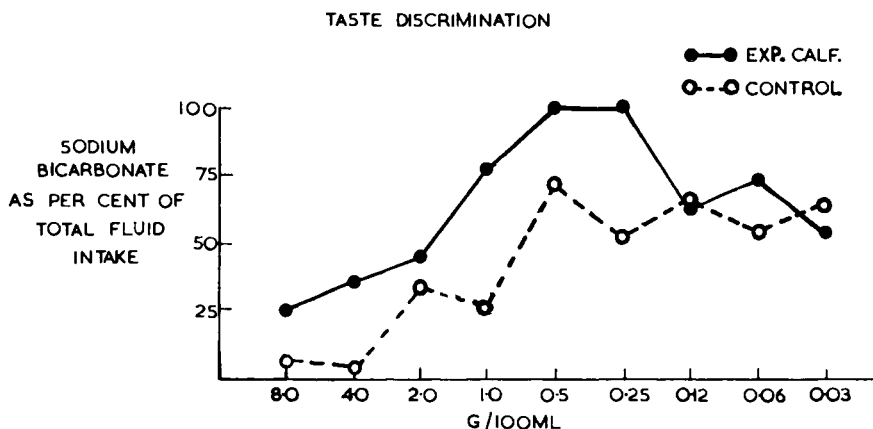
FIG. 3. Two-choice preference test for sodium bicarbonate in monovular twin calves one of which was sodium depleted. (Bell and Williams, unpublished data.)

When offered falling dilutions of sodium salts the experimental calf at first increased the intake of saline solution so that the Na/K ratio in the saliva remained normal. The animal appeared to be responding to some internal regulatory mechanism of the kind postulated by Richter (1943). When, however, the saline solutions became very dilute the animal failed to take in a sufficient volume of sodium salt and consequently became sodium depleted (Fig. 4). Apparently the self regulatory process collapsed

at the extreme dilution range possibly due to an effect on the whole animal because of the disruption of its sodium economy. On the other hand the threshold for salt taste may have been raised in the experimental animals so that the receptors were unaffected by the dilute saline.

THE EFFECT OF FEEDING Na⁺ SUPPLEMENT ON SALIVARY Na⁺ AND K⁺ CONCENTRATIONS
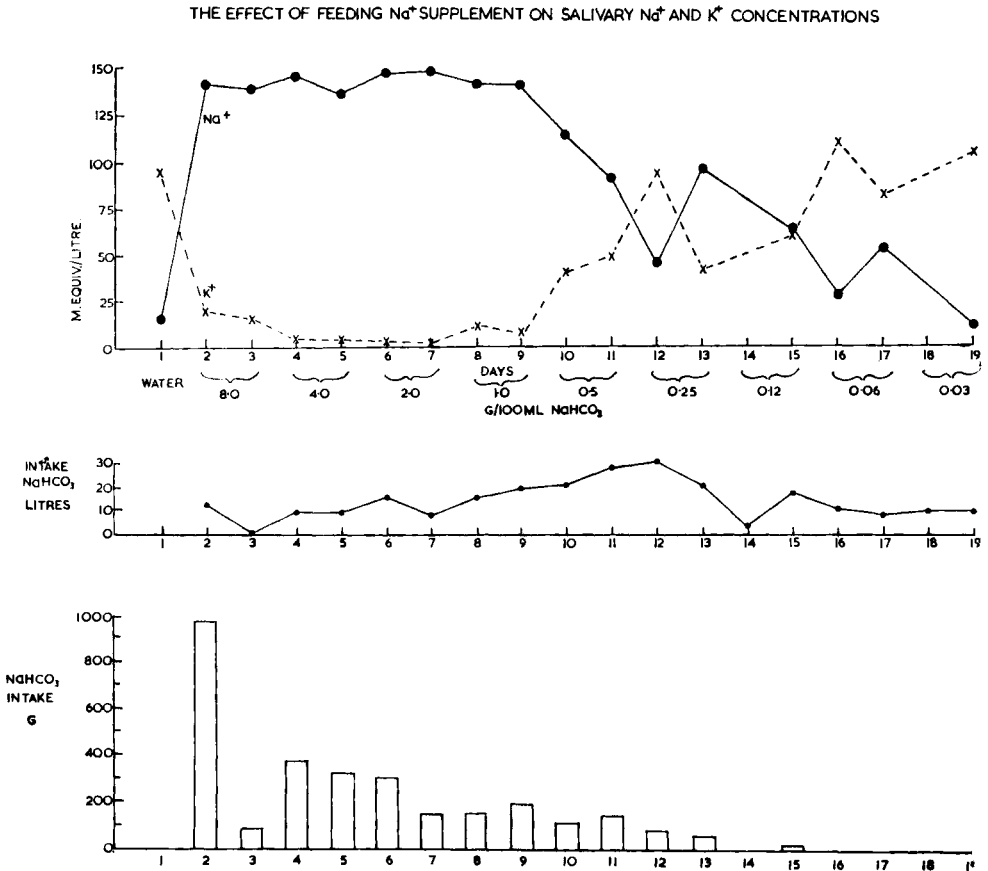
FIG. 4. Shows the effect on salivary Na+ and K+ when a sodium depleted calf is allowed the choice between sodium bicarbonate and water. (Bell and Williams, unpublished data.)

Behavioural changes become very obvious in calves made sodium depleted through loss of saliva to the exterior. If salt from a tin is placed on the ground or salt solutions are prepared within view of a sodium depleted animal it will show much motor activity and become quite agitated and make attempts to gain access to the saline material. A normal calf on the other hand is indifferent to these procedures. Furthermore, if a sodium deficient calf is allowed to select from a series of solutions which are known to be sapid for calves contained in identical containers it will search until

it finds the saline solution and immediately begin to drink. This type of self-selection behaviour provides further support for Richter's view and indicates that the sense of taste can play an important part in the " environmental homeostasis " of the ruminant herbivores. Recent work in sheep and cattle suggests that the Na/K ratio of saliva is a variable quantity which may be reflecting fluctuation in the sodium status of the animal (Sellers and Dobson, 1960 ; Bailey and Balch, 1961). The rise in the potassium content of the saliva results from increased secretion of adrenal corticosteroids due to sodium depletion (McDonald and Reich, 1959). Although the adrenal gland is the main source of the controlling mechanism of electrolyte control in sodium depleted sheep it is possible that other areas, perhaps some part of the central nervous system may also be concerned (Coghlan, Denton, Goding and Wright, 1960).

It becomes apparent, therefore, that in ruminants there is a close interrelationship between taste, metabolism and nutrition. At present the experimental data are sparse but the availability of a good experimental preparation augurs well for future investigations.

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SOME THALAMIC AND CORTICAL MECHANISMS OF TASTE

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THE first and major part of this paper will describe the thalamic taste systems of the rat and squirrel monkey. Later, there will be some speculations about the cortex.

The research on the thalamus utilized two electrophysiological techniques. The first mapped the thalamic projections of three tongue nerves, the chorda tympani and the lingual branch of the IXth, both known to contain taste fibers, and the lingual branch of the Vth which is presumably devoid of taste. The nerves were stimulated electrically while the thalamus was probed with a large microelectrode. The slow components of the evoked response were attenuated by filters leaving a multi-unit burst of spike discharges as the criterion response. Such maps do not give any information about the functional characteristics of the system or for that matter, cannot even identify the modalities involved, but they do provide an indispensable guide for the second stage, microelectrode recording from single units using adequate stimulation. The single unit technique provides the only definitive localization and one form of information about the functional characteristics of the system.

Figure 1 shows the thalamic projection of the three tongue nerves in the rat (Emmers, Benjamin and Blomquist, 1962). The electrode tracks are reconstructed on six standard diagrams, most anterior at the top, most posterior at the bottom. The broad terminal bars on the tracks mark the extent of response, which in these experiments was a multi-unit discharge recorded with a large microelectrode. The oval-shaped, morphologically distinct subnucleus of the ventralis is outlined in the middle 4 diagrams.

A comparison of these three maps reveals one important relationship. The projection of the lingual nerve, presumably tasteless, is located more laterally in the subnucleus than the projection of the two taste nerves. They fill the most medial part. This spatial separation of nerve responses suggested the possibility that there might also be a spatial separation of modalities. Taste might project exclusively to this medial tip and have its own nuclear territory independent of touch, temperature and other modali-

FIG. 1. Electrode tracks from three types of nerve preparations constructed on standard cross-sections at 6 successive levels of the rat's thalamus. Most anterior level at the top (No. 1), most posterior at the bottom (No. 6), spaced at 0.2mm intervals. The broad terminal bars mark the extent of response. CM ? = n.centrum medianum ?, MD = nc. medialis dorsalis, PF = nc. parafascicularis, PV = nc. paraventricularis, SPF = nc. subparafascicularis, THP = habenulointerpenduncular tracts, V = nc. ventralis. From Emmers, Benjamin and Blomquist, 1962.

ties. This possibility was appealing, but did not seem compatible with the fact that taste, touch, and temperature units were all found intermingled in the somatic sensory tongue area in the cortex of Swedish cats (Cohen

• TASTE   ○ COLD

× MECHANICAL   △ WARM

FIG. 2. Localization of electrophysiologically isolated single neurons in the rat thalamus responsive to stimulation of the tongue. Abbreviations as in Fig. 1.

et al., 1957 ; Landgren, 1958). The thalamus of American rats proved to be different, however. Frommer recorded multi-unit responses to taste stimuli only in this medial location showing that taste was not coextensive with the other modalities (Frommer, 1961 ; Frommer and Pfaffmann, 1961 ; Pfaffmann *et al.*, 1961). The results of a single unit study shown
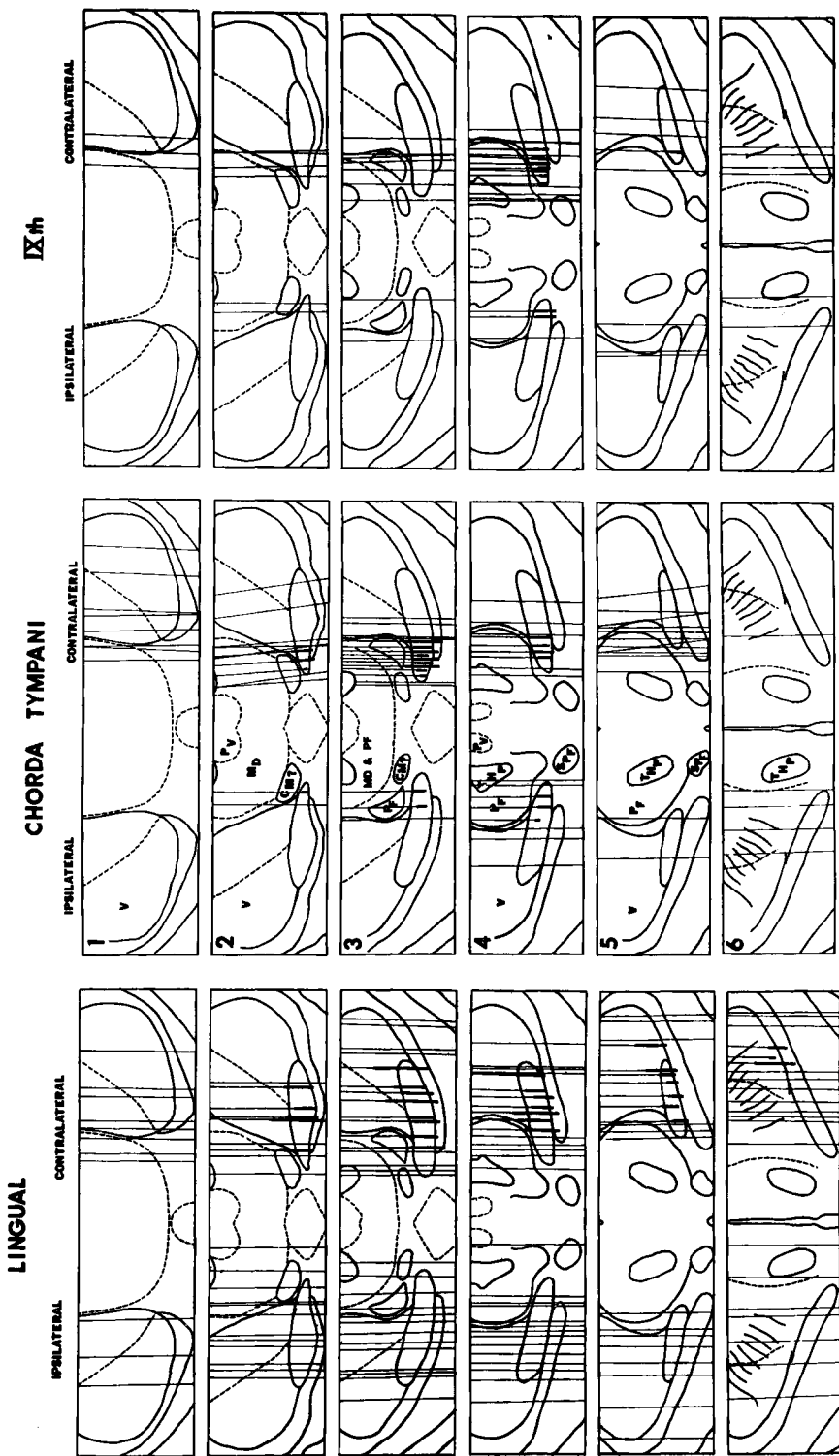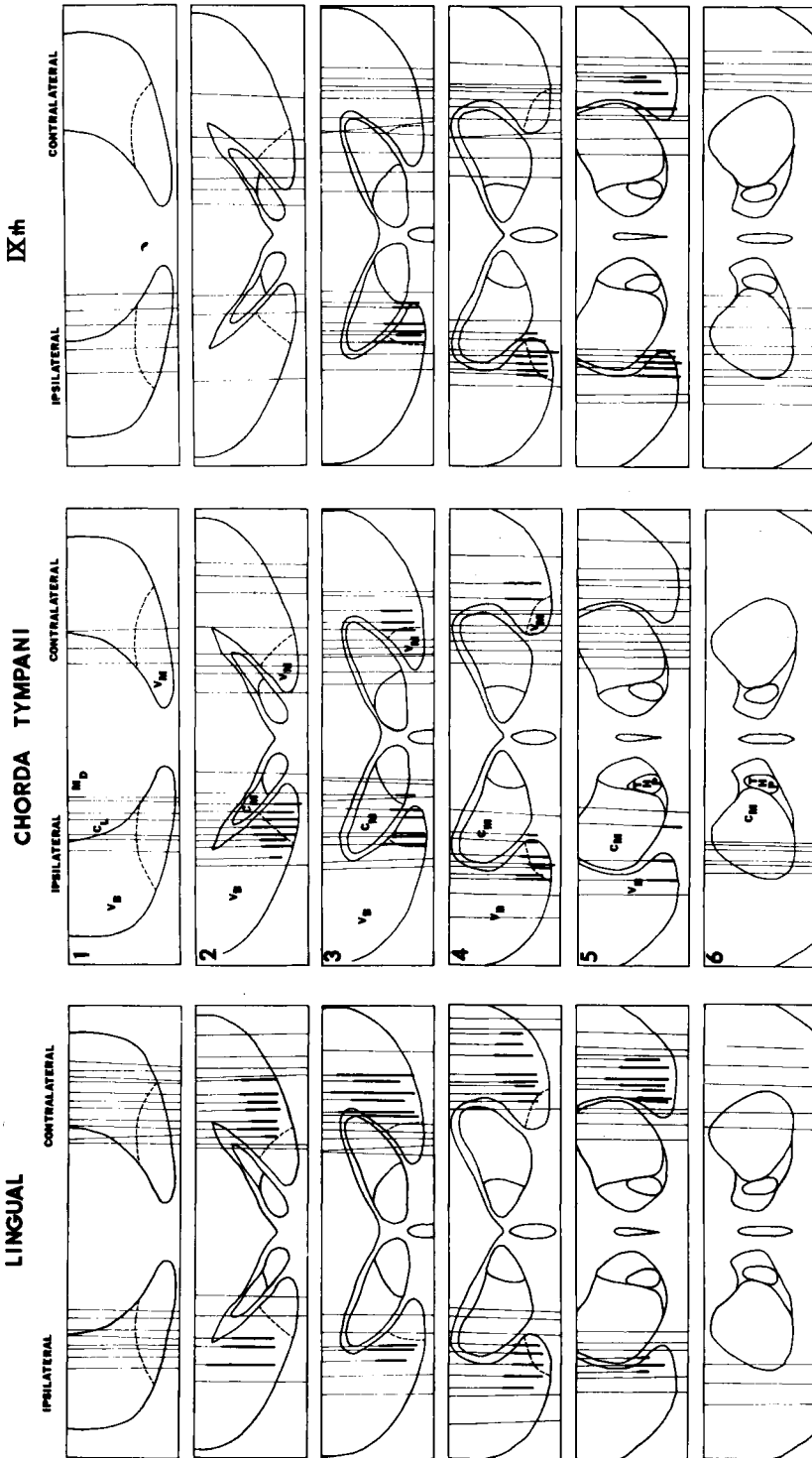
FIG. 3.   Electrode tracks from three types of nerve preparations reconstructed on standard coronal sections at 6 successive levels of the squirrel monkey thalamus.   Most anterior level at top (No. 1), most posterior at bottom (No. 6) spaced at 0.45 mm intervals.   The broad terminal bars mark the extent of responses.   Vertical lines with no bars indicate negative punctures.   Dashes mark lateral border of ventromedial complex.   CL = n. centralis lateralis ;   MD = n. medialis dorsalis ;   THP = habenulo-interpeduncular tract ;   VB = ventrobasal complex ;   VM = ventromedial complex.   From Blomquist, Benjamin and Emmers, 1962.

in Fig. 2 clearly established that the taste neurons were spatially independent (Blomquist, Benjamin and Emmers, 1962). The location and modality identification of these single neurons are plotted on the same

IPSILATERAL   **CONTRALATERAL**

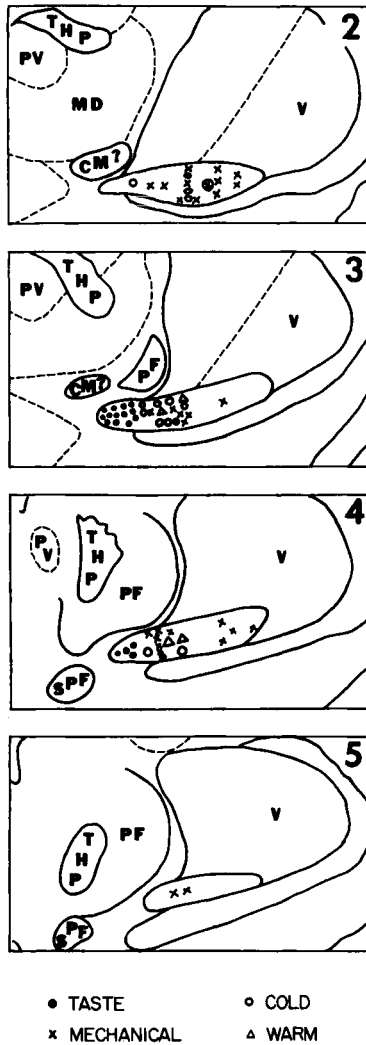● TASTE   ○ COLD   × MECHANICAL   ⊕ MECH. & COLD
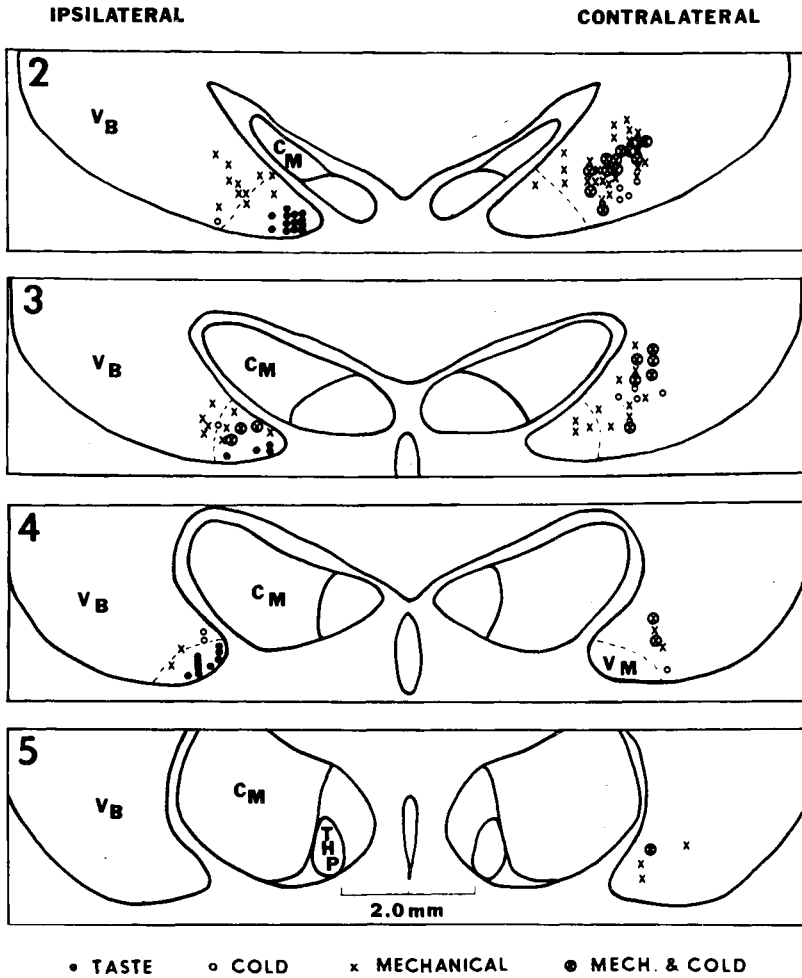
FIG. 4. Localization of electrophysiologically isolated single neurons in the squirrel monkey thalamus responsive to stimulation of the tongue. Abbreviations as in Fig. 3.

standard diagrams, this time ignoring laterality. Notice that the taste units (solid circles) are located medially, by themselves, while units responsive to mechanical stimulation or to temperature or both are mixed in a more lateral location.

The same two-stage sequence has been completed for the squirrel monkey (Blomquist, Benjamin and Emmers, 1962; Benjamin and Blomquist,

1962). The projections of the tongue nerves (Fig. 3) are more complex in this animal, but only a few points need be mentioned for this discussion. On the ipsilateral side the relationships are much like those in the rat. Anteriorly, there is a place for taste medial to the lingual nerve projection. On the contralateral side, however, there seems to be no place for an independent taste area. Not only is there complete overlap of all the responses, but the taste nerve projections do not even reach the medial tip of the nucleus. These data would seem to preclude spatial independence for taste in the squirrel monkey unless the taste input turned out to be purely ipsilateral in this animal. This eventuality seemed unlikely in view of the bilaterality of effects from human cortical lesions (Bornstein, 1940). Nevertheless, a subsequent single unit analysis (Fig. 4) established that taste was, indeed, ipsilateral. Notice that all the taste units (solid circles) have been plotted in the ipsilateral ventromedial nuclear complex. This laterality was conclusively established for all except five of the units which had receptive fields on the extreme back of the tongue. Independence is maintained, but unlike in the rat, the separation shifts from a medio-lateral plane anteriorly, to a dorso-ventral plane posteriorly.

It is clear, then, that in both the rat and squirrel monkey thalamus the taste system is spatially separated from other tongue modalities.

Thus far taste units have been discussed as if they responded exclusively to taste stimuli. Actually, this was not the case, especially in the rat (Table 1). All of the units tested (13 of the total 18) also fired when extremely cold water was flowed on the tongue. The response of one unit is shown on a frequency vs. time plot in Fig. 5. The stripped bar shows the resting

TABLE 1. SUMMARY OF UNITS LOCALIZED IN THE THALAMUS OF THE RAT AND SQUIRREL MONKEY. ONLY 13 OF THE 18 TASTE-TEMPERA-TURE UNITS IN THE RAT WERE ADEQUATELY TESTED FOR A TEMPERATURE RESPONSE.

	Squirrel monkey	Rat
Taste	24	—
Taste and temp.	3	18*
Cold	13	13
Warm	—	4
Mechanical	59	28
Mech. and temp.	26	2
Total	125	65

activity of the unit at 2 to 3 impulses/sec. The unit responded to a " white " taste solution (an atrocious mixture of quinine, sodium chloride, hydro-chloric acid, and sucrose) in a slowly adapting, tonic fashion. The unit

also responded to 12°C water, but only phasically. This duplicity of res-
ponse probably does not represent a convergence of modalities at the
thalamus for single peripheral taste fibers in the rat behave in a similar
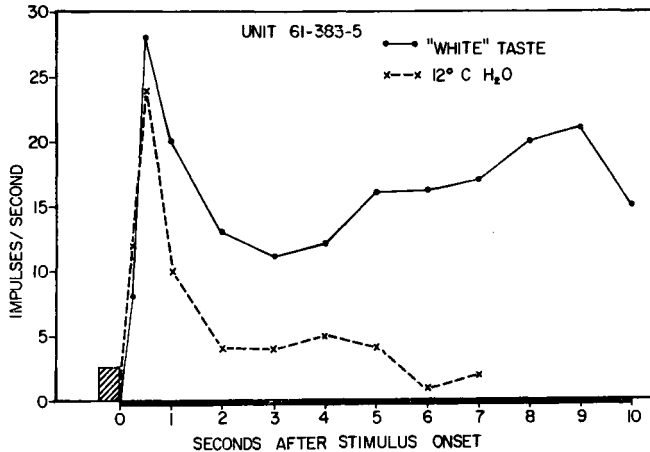fashion (Fishman, 1957).

FIG. 5. Frequency plot of the responses of a single thalamic neuron in the rat to
solutions flowed onto the tongue.

Most squirrel monkey taste units did not show this duplicity of response
(Table 1). Only three of twenty-seven taste neurons responded to tem-
perature stimuli.

Species differences in the response of taste neurons to taste solutions are
not uncommon, just difficult to explain. But why should taste neurons
respond to temperature in one animal and not in another? One cannot
attribute the separation of taste and temperature to a general phylogenetic
superiority for the squirrel monkey mixes some modalities as well. This
animal had a large proportion of " mechanical " neurons which also res-
ponded to temperature whereas the rat tended to keep these separate.

As in the case of the rat taste units, the bimodality of the mechanical-
temperature units in the squirrel monkey probably does not represent
convergence at the thalamus. Hensel and Zotterman (Zotterman, 1959)
have described similar bimodal single fibers in the lingual nerve of the cat
and have shown that their response to temperature stimuli is quite dif-
ferent from the response of unimodal temperature fibers. The same
difference is characteristic of these squirrel monkey thalamic neurons. I
would like to show you a typical sample of each which is truly typical. The
responses will be presented in the form of interspike interval dot patterns,
a unique and powerful method of data representation described by Wall
(1959). First, for purposes of clarification let me interject Fig. 6 which

contrasts interspike interval plots with the more familiar frequency plots. These are examples of " nociceptive " unit responses which are particularly well suited to illustrate the characteristics of dot patterns.

At the upper left is a frequency plot of a common, slowly, adapting unit. It has no spontaneous activity. When the stimulus is applied (black bar), the frequency of spikes increases rapidly to a maximum and then slowly decreases as the stimulus continues over 60 sec. At the termination of the
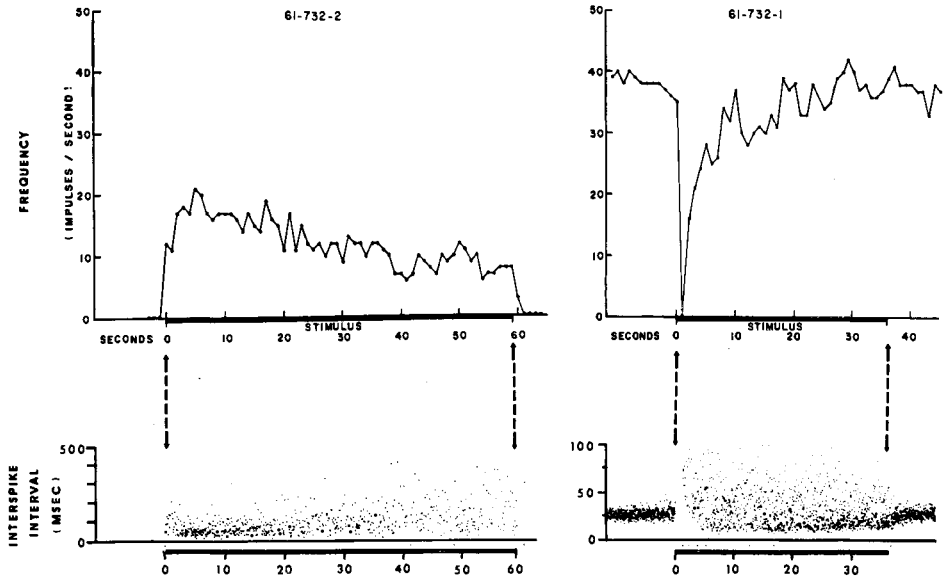

FIG. 6. Comparison of frequency plots and interspike interval dot patterns for two single neurons. See text for explanation.

stimulus, the frequency of impulses again returns to zero. Below is an interspike interval plot of the same response constructed on the same time scale. The ordinate, however, measures the interspike intervals, that is, the time between two successive spikes. Each dot represents a spike and its distance from the abscissa (0 msec) indicates the amount of time in msec. since the previous spike. A short time corresponds to high frequency, a longer time, to a lower frequency. This plot is photographed from taped data being played back to an oscilloscope which is sweeping up the ordinate, so to speak. The circuitry is arranged so that the first spike triggers the sweep, in this example 500 msec in duration. The second spike does several things ; it intensifies the beam to produce a dot, immediately returns the sweep to zero, and then triggers another sweep. The next spike produces another dot marking the second interval, resets the sweep and so on. These dots are photographed on very slowly moving film which spreads them out along the abscissa. Although it is not apparent, there is

only one dot per sweep and each dot, therefore, measures a single interval.

At the beginning of the stimulus this unit fires fairly regularly with a short interspike interval averaging about 50 msec in length which corresponds to a frequency of about 20 impulses/sec. As the stimulus continues the average interval between spikes increases and also becomes more variable as shown by the increased scattering of the dots.

At the right is an example of a unit which is inhibited by stimulation. It has a fairly regular spontaneous discharge which is completely inhibited during the first few seconds of stimulation. After about 10 or 15 sec of continuous stimulation, the average interval returns to normal but the regularity of firing is affected to the end of the stimulation period.

Clearly, these interspike interval dot patterns have at least two important advantages. First, they present all the data in a perceptually convenient and meaningful form and secondly, they construct themselves automatically without tedious human labor.

After this lengthy introduction, examples of the two types of temperature units are finally presented in Fig. 7. At the top is a dot representation of
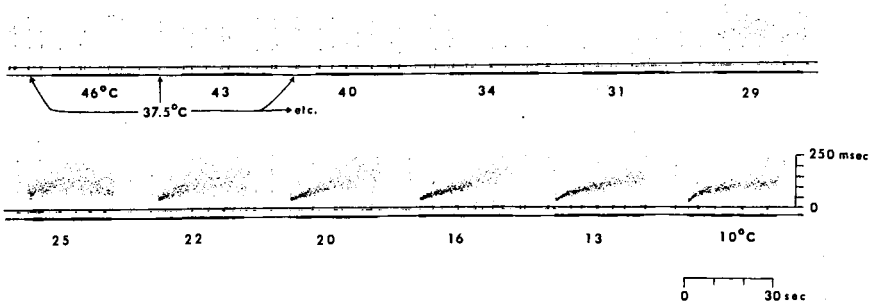
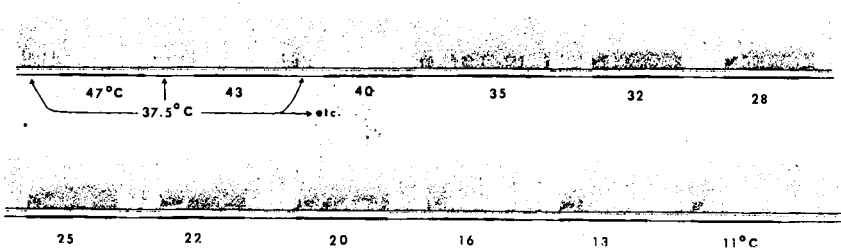FIG. 7. Interspike interval dot patterns of the responses of two different kinds of thalamic neurons to solutions of various temperatures flowed onto the tongue of the squirrel monkey.

the response of a bimodal unit. The record is continuous, representing about nine minutes of stimulation allotted as follows: water at body temperature (37.5°C) flowed onto the tongue for 15 sec, followed a test

stimulus of 46°C for 30 sec, back to body temperature for 15 sec, another test stimulus (43°C) for 30 sec, and so on. The unit had very little spontaneous discharge, began to respond at about 29°C and began to display its characteristic pattern at about 22°C which consisted of an initial discharge at short intervals with low variability. As the stimulus continued, both the average interval and the variability increased.

The unimodal temperature units fired quite differently. At the bottom is one example. Notice that it always gave a burst of impulses to a colder stimulus, no matter what the absolute temperature. Look at the change from 43°C to 37.5°C and from 37.5°C to 11°C. In its optimal temperature range (32°–22°) this initial burst was followed by a discharge at relatively constant range of intervals.
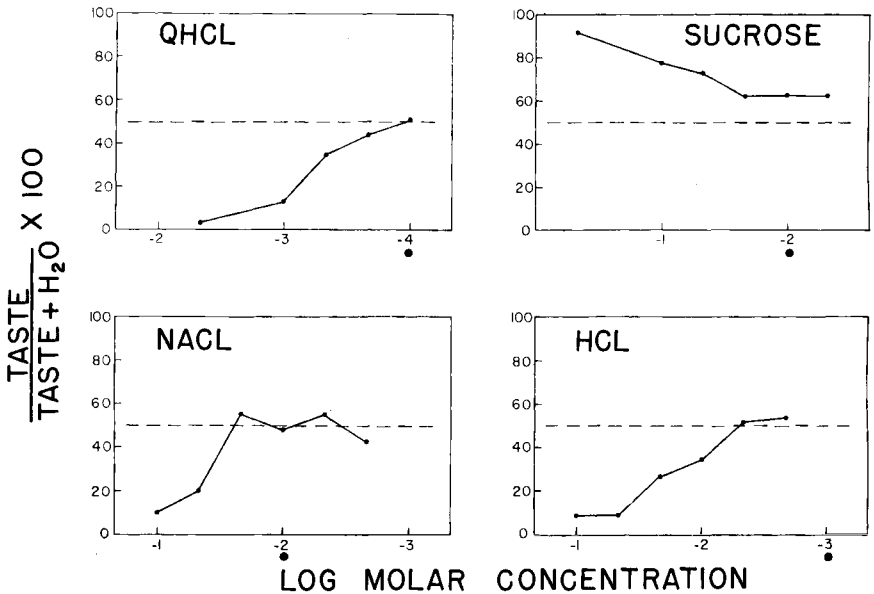
FIG. 8. Taste preference curves of the squirrel monkey as determined by the two bottle method. Large dots indicate the lowest concentrations used in the electrophysiological experiments.

These two patterns of discharge are sufficiently characteristic that one can always identify the modality of a unit from the record.

The taste units in the squirrel monkey were routinely tested with mechanical and temperature stimuli and then subjected to a series of taste solutions which consisted of three concentrations each of four old standards : sodium chloride, hydrochloric acid, quinine hydrochloride, and sucrose. The three concentrations were chosen on the basis of behavioral discrimination thresholds as measured by the standard two-bottle preference method (Fig. 8). These are average curves based on the consumption of four

animals, two descending runs each. The lowest concentration marked by the large dots was below threshold, the second was 10× stronger and the third 10× stronger than the second, placing it in each case well into the range of maximum preference or aversion. I should mention that the squirrel monkey does show a preference for NaCl on an ascending series, but these data are not complete as yet.

It was very difficult to maintain isolation of these extremely small and densely packed cells for very long periods. Therefore, each stimulus application was limited to about three seconds, but all solutions were presented a sufficient number of times to be certain of the result.

Figure 9 shows the response profiles for 27 units. Each profile shows

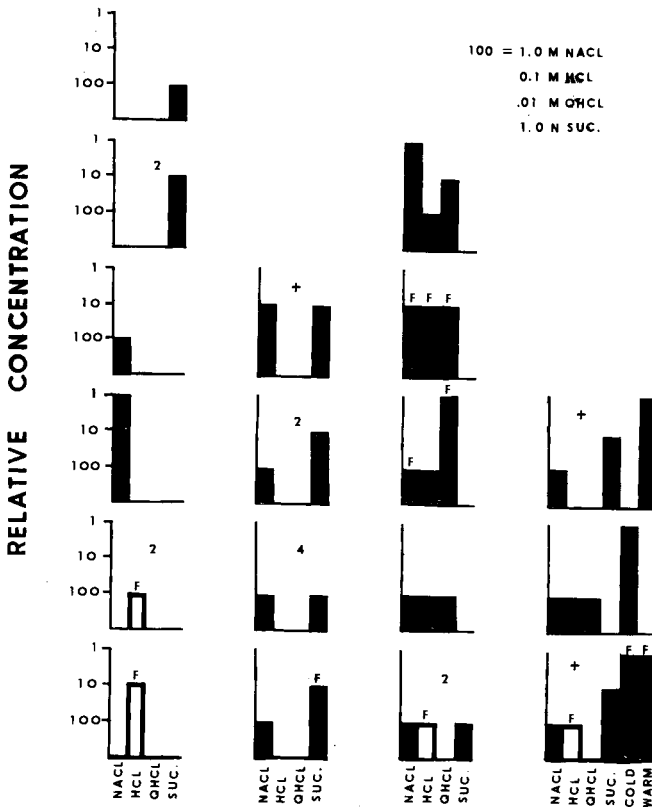FIG. 9. Response profiles for twenty-seven thalamic neurons isolated in the ventromedial nuclear complex of the squirrel monkey.

whether or not the unit was activated by a particular substance at a particular concentration, but does not indicate the magnitude of the response. A number specifies how many units had the same response profile. In the column at the extreme left are profiles for eight units which were responsive

to only one substance. For example, the profile at the top represents a unit that responded only to the strongest concentration of sucrose. Notice the NaCl units and in the bottom two profiles some units that did not respond to any taste solution, but did fire to a water rinse after HCl. These " off " responses are indicated by open bars and the letter " F ". The next column contains the profiles of eight units responsive to two substances, in all cases, NaCl and sucrose. Notice the on-off response of the bottom unit. The plus sign signifies a unit of positive polarity, with spikes of extremely short duration and extremely small amplitude. In the folklore of indium microelectrode enthusiasts these characteristics suggest pickup from a fiber.

In the next column are six units activated by three substances each including quinine and finally, in the last column three units driven by temperature as well as taste stimuli. Notice that a response to quinine was always associated with a response to both HCl and NaCl and never with a response to sucrose.

On Fig. 10 are sample dot patterns of units subjected to stimuli of longer

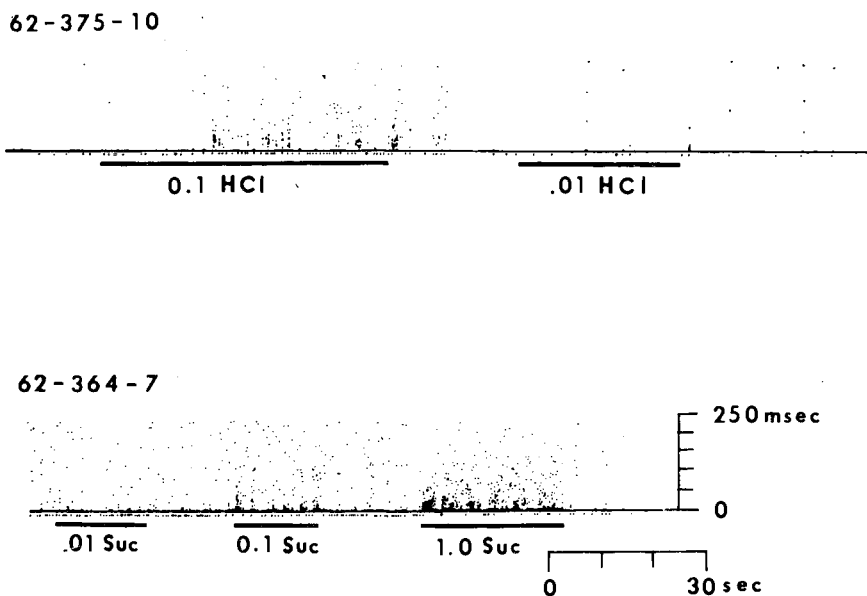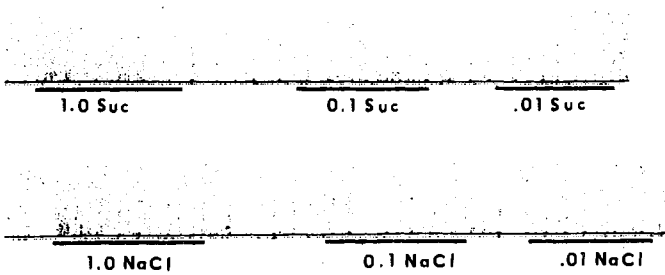FIG. 10. Interspike interval dot patterns of the responses of two thalamic neurons to taste solutions flowed on to the tongue of the squirrel monkey. Heavy bars mark periods of stimulation.

duration. The one at the top is an HCl " off " unit which did respond to HCl after about 20 sec of continuous stimulation. The " off " burst so prominent after a 3 sec stimulus is attenuated but still evident. The

bottom plot shows a unit which responded to the two strongest concentrations of sucrose. You can see why these dot patterns were shown after the temperature units. The patterns are not distinctive, for most of these thalamic taste units were very poor responders.

At the top of Fig. 11 is a plot of a unit which discharged to both sucrose and sodium chloride. Again the meager response is evident. Contrast this with the vigorous response of the unit at the bottom. He was a positive fellow, isolated in the same puncture and activated by the same solutions. If you are willing to entertain the notion that this positive unit is

62-375-20

1.0 Suc   0.1 Suc   .01 Suc

1.0 NaCl   0.1 NaCl   .01 NaCl

62-375-15

1.0 Suc   0.1 Suc

250 msec

0

1.0 NaCl   0.1 NaCl

0   10 sec
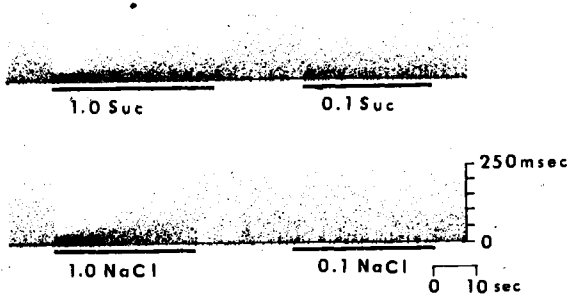
FIG. 11. See Fig. 10.

indeed a fiber, and if you are willing to contemplate the possibility that he may represent the input to thalamic cells such as the one above, then something drastic happens at this synapse. The output is severely reduced. Perhaps this attenuation is due to the anesthetic (nembutal), perhaps it is normal, but if it is repeated at subsequent synapses, at the cortex for example, there would not be much response left to record.

At least one generality is clear from these data. The sensory code at the thalamus is complex. Other generalities are not immediately apparent.

Now I would like briefly to consider the cortical taste system, first, in the

rat where the results, at least at one time, seemed clear and consistent, and secondly, in the squirrel monkey where they are not.

The results of electrical stimulation of the nerves in the rat are summarized in Fig. 12 (Benjamin and Pfaffmann, 1955). The evoked responses, slow waves recorded from the brain surface with a gross electrode, were confined to these two small areas, one for each nerve. Maps from six of these experiments were combined to produce the composite area outlined in the lower diagram.
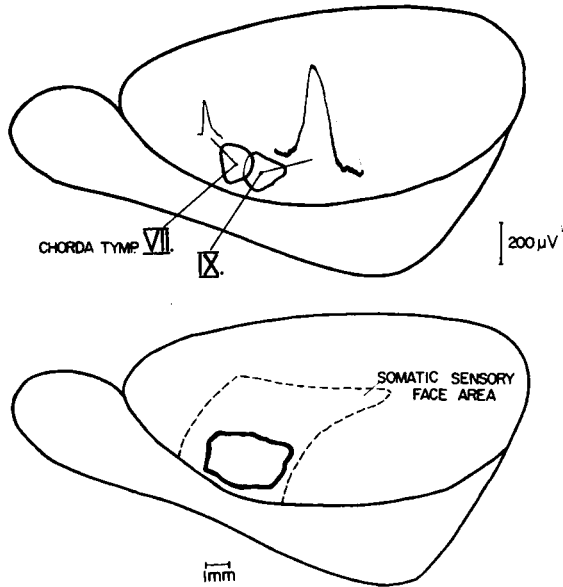
FIG. 12. Above : Cortical areas in the rat activated by electrical stimulation of chorda tympani and the lingual branch of the IXth nerve. Below : " the composite taste nerve area " compiled from six such recording experiments. From Benjamin and Akert, 1959.

The gustatory function of this composite taste nerve area was established by ablation experiments. Bilateral removal of the composite area produced impairment of taste discrimination as measured both by preference (Benjamin and Pfaffmann, 1955; Benjamin and Akert, 1959; Benjamin, 1959) and conditioning techniques (Benjamin, 1960). Conversely, removal of all of the rest of the neocortex sparing the composite area left discrimination normal. These two types of lesions localized taste impairment to the composite area. Further parcellation established that the ventral part of this total area was the most crucial for normal taste behavior. Some sample lesions and the associated thalamic degeneration diagrammed in Fig. 13 illustrate this point. At the top is a lesion of the whole composite area which produced degeneration of the whole subnucleus of the ventralis

and, of course, a taste deficit. The dots emphasize that the degeneration was not complete ; many normal cells remained throughout the degenerated area.   If the lesion spared the ventral part of the composite area (cases 2 and 4) discrimination remained normal and so did the medial part of the subnucleus.   Conversely, a ventral lesion (case 3) was associated with a taste deficit and medial degeneration.
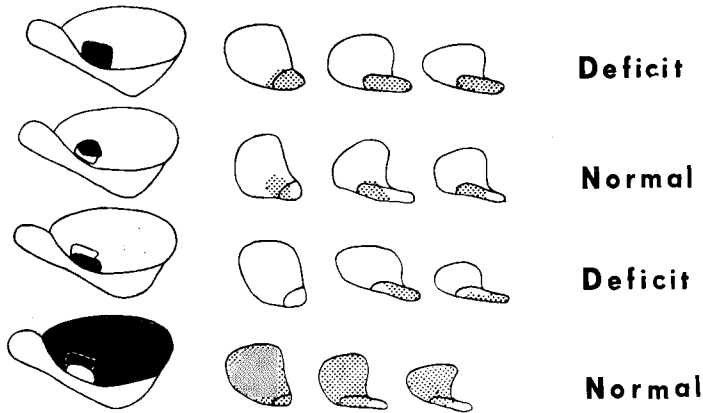
FIG. 13.   Diagrams of some sample cortical lesions in the rat, the associated retrograde thalamic degeneration, and postoperative status of taste discrimination.   The composite taste nerve area is outlined.   All lesions were bilaterally symmetrical.

All of the data from the rat fit together rather well.   The ventral part of the taste nerve area, which is most crucial for taste behavior, receives projections from the medial part of the thalamus where, as we have seen, microelectrode studies have localized the taste cells.   I should add that severe taste impairment results from thalamic lesions placed directly in this medial part (Ables and Benjamin, 1960 ; Benjamin, 1960 ; Oakley and Pfaffmann, 1962).

Finally, to complete the picture, this localization of cortical taste function in the American rat is quite compatible with the localization of the taste units in the cortex of Swedish cats (Cohen *et al.*, 1957 ; Landgren, 1958).

We would still be complacently happy had we not tried the same type of experiments on the squirrel monkey.   Lesions of the taste nerve areas in this animal do not produce consistent taste impairment nor do they produce degeneration of the proper part of the thalamus.

Figure 14 shows the evoked potential areas of the various taste nerves in the squirrel monkey.   Electrical stimulation of the chorda tympani nerve (Benjamin and Emmers, 1960) produced not one area, as in the rat, but two areas.   The posterior one was situated right in the middle of the lingual nerve area though this is not illustrated on the figure.   The other chorda area, drawn in black, was situated anterior to the lingual nerve projection

and, in fact, completely outside somatic sensory cortex (Benjamin and Welker, 1957). This spatial separation reminded us of the rat thalamus and again led to the enticing possibility that the anterior area (black) might be pure taste. The results from the IXth nerve were encouraging. Again there were two projection areas, one of which was assigned to taste
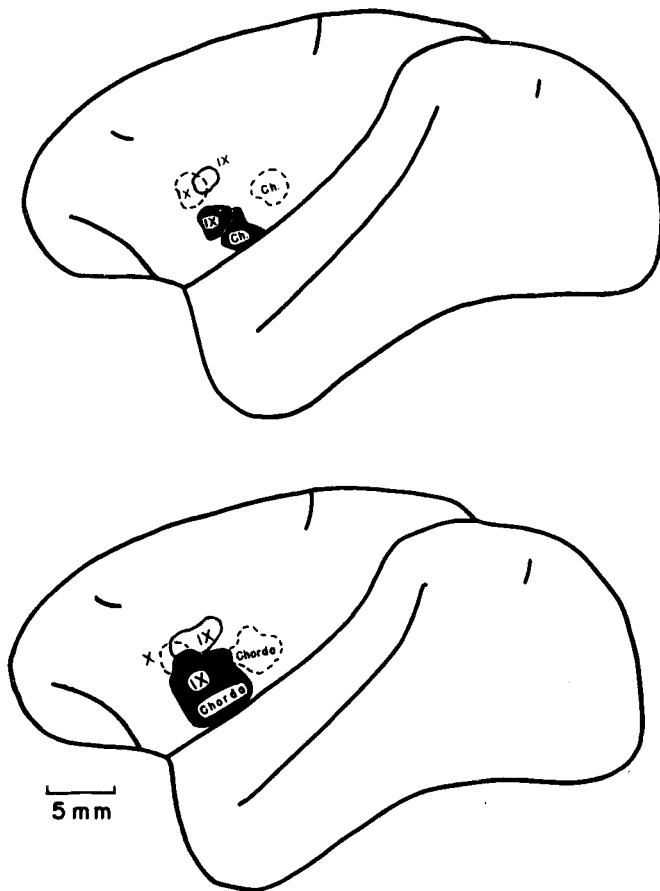
Fig. 14. Above : Cortical areas in the squirrel monkey activated by electrical stimulation of the chorda tympani, lingual branch of the IXth and pharyngeal branch of the Xth nerves. Below : Composite area compiled from a number of such recording experiments.

(black). Stimulation of the pharyngeal branch of the Xth nerve which has few, if any, taste fibers, conveniently produced only one responsive area. Our conclusion was that the black areas had taste function and the white areas constituted the non-gustatory somatic sensory tongue properly oriented with its tip pointing into the mouth (Woolsey, 1958).

Maps from a number of experiments were combined to produce the

lower diagram which served as a guide for lesions. If our hypothesis was correct, ablation of the black cortex should produce taste deficits and degeneration of the thalamic taste cells in the ventromedial complex.

The situation no longer seems to be black and white. Thus far, Blomquist and I have removed the black area in five monkeys and produced only one small taste deficit, removed all of the areas both black and white in three animals and produced no deficits, and finally, in three animals, removed all of these areas and in addition, the fronto-parietal operculum and part of the insula producing only one slightly deficient monkey. This was disturbing. The typical degeneration picture, as seen in Fig. 15, was even
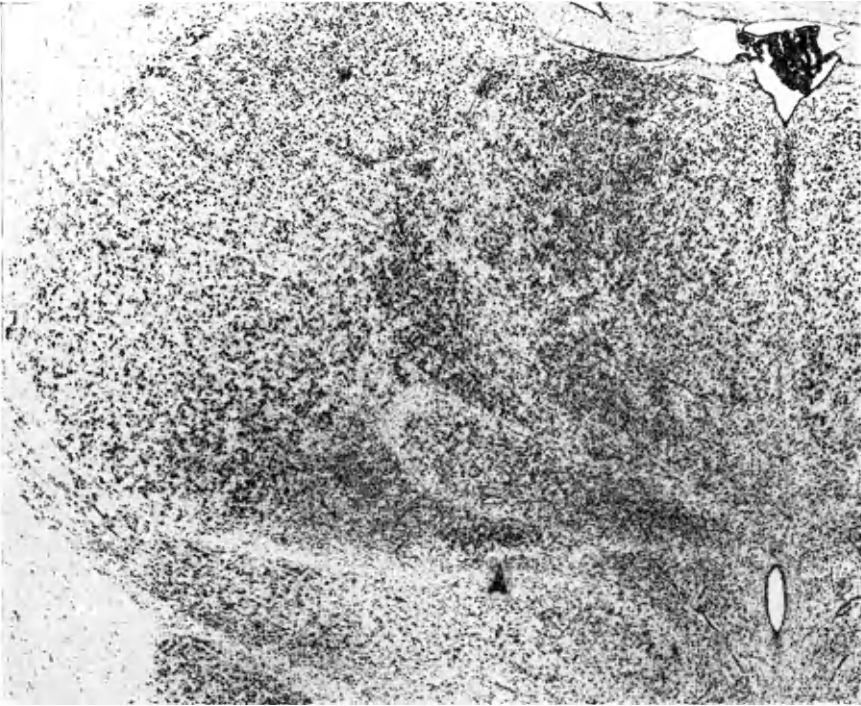
FIG. 15. Retrograde degeneration in the ventrobasal nuclear complex of the squirrel monkey resulting from the cortical lesion in Fig. 16. Arrow marks some undegenerated cells in the posterior ventromedial nuclear complex presumably belonging to the taste system.

more disturbing. The degeneration extends from the lateral border of the centrum medianum toward the tip of the ventromedial complex, but leaves a little clump of normal cells right where the microelectrode results would locate taste. Not only did these cortical lesions fail to produce consistent taste impairment, but they apparently did not produce degeneration of all

the thalamic taste cells. What does this mean? If these are indeed taste cells, then they must project to some area not removed by the lesion (Fig. 16). On the surface the ablation marked by black included all of the evoked potential taste nerve areas (black and white) and within the fissure, most of the fronto-parietal operculum and underlying insula as well. The right
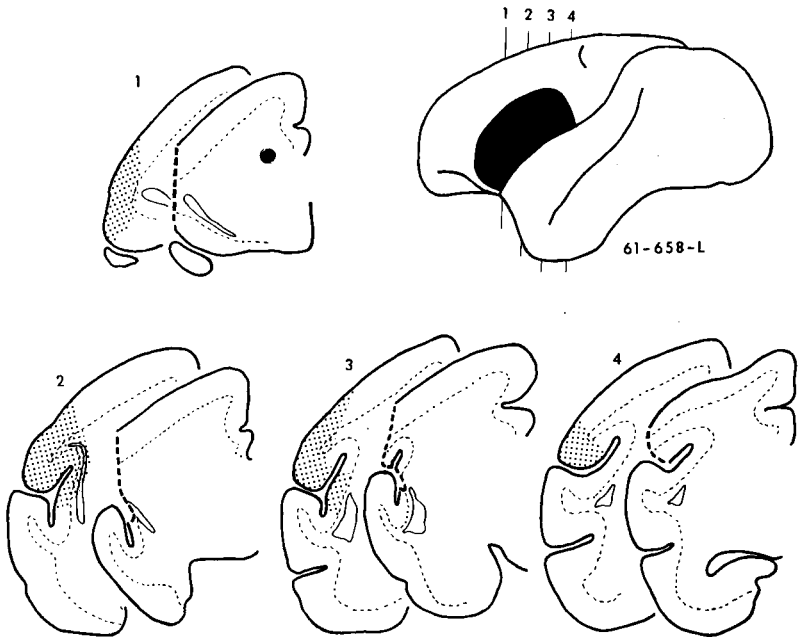
FIG. 16. Reconstruction of a cortical lesion in one hemisphere of a squirrel monkey producing the degeneration in Fig. 15. The lesion was approximately bilaterally symmetrical. The right section of each pair is from the experimental brain, the left section from a normal brain. The dots approximate the removed tissue. The claustrum is outlined.

section of each pair is from the experimental brain, the left an equivalent normal section on which dots indicate approximately the tissue that was removed.

Where is the projection of those intact taste cells? Probably not to the intact cortex on the surface ; first, because most of this is known to be occupied with other functions, and secondly, because other investigators could not produce taste deficits with even much larger surface lesions (Patton, 1955). They were able, however, to produce substantial deficits in taste discrimination with lesions inside the Sylvian fissure involving oper-cular and insular cortex (Bagshaw and Pribram, 1953 ; Patton, 1955). Unfortunately, in the squirrel monkey most of the remaining opercular and insular cortex belongs either to the trunk part of the somatic sensory

area II or to the auditory system (Benjamin and Welker, 1957 ; Hind *et al.*, 1958).

Where then? I would like to suggest the claustrum as a possibility. There is really no substantial evidence for this speculation, but none against it either. The claustrum lies in a very vulnerable position (outlined area) and in fact, was partially damaged by this lesion (cross section 2).

In the rat the claustrum is in an even more vulnerable position (Fig. 17) because it has not split off from the rest of the cortex and lies directly under

FIG. 17. Coronal section of a rat brain through a unilateral lesion removing the composite taste nerve area. The dark-celled mass subjacent to the lesion is the claustrum.

the ventral part of the composite taste nerve area. Cortical lesions which produce taste deficits always damage this dark-celled claustral mass and properly so since it is part of the cortex.

The speculation that there are gustatory projections to the claustrum may not be substantiated. However, the conclusion that the thalamic taste projections are not confined to the cortical area defined by evoked potentials seems inescapable.

In summary, although it appeared at one time that the cortex was the known and the thalamus the unknown, the situation is now reversed.

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NATURAL CONDITIONED SALIVARY REFLEX OF MAN ALONE AS WELL AS IN A GROUP

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INTRODUCTION

KERR (1961) believes, " the commonly held belief that salivation is pro-voked by stimuli to which individuals have become conditioned—for example, the thought or sight of food—does not appear to be supported by any objective studies reported in the literature ". He repeated attempts to elicit any existing conditioned salivary reflexes by presentation of the idea of food, and the sight of lemons and candy was unsuccessful. Thus he was inclined to deny a natural conditioned reflex in man. On the other hand, Hayashi and Suhara (1962) have shown that the artificial conditioned salivary reflex can be established. The method was to measure salivary secretion through a suction cup which was attached to the orifice of the parotid gland of the subject (Lashley, 1916).

When we do experiments with a suction cup, a parotid gland secretes at fairly steady rates which are characteristic for the individual. This saliva is called "resting saliva", and the established conditioned saliva, of course, exceeds the resting value.

First, a trial was made to evaluate the natural conditioned saliva by the suction cup method in Japanese adults. The persons who were used in the first experiment were between 18–27 and 9–10 years old. The adults were all students of higher schools or universities in Tokyo and the children were boys and girls at primary school.

DRAWINGS ELICITED NO NATURAL CONDITIONED REFLEX EITHER IN THE ADULT OR CHILD

Pictures of biscuits, apples and oranges were placed before the subjects while the resting saliva was measured. The saliva did not increase nor decrease. In other words, pictures elicited no natural conditioned reflex within these age-groups.

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THE AMOUNT OF NATURAL CONDITIONED REFLEX WAS DETERMINED ACCORDING TO THE STRENGTH OF THE UNCONDITIONED STIMULUS

Biscuits, apples, oranges and pickled plums, usually favourites of Japanese in everyday life, were shown to adult subjects. The parotid saliva increased over the resting range. One of the results is shown in Table 1. Some of the subjects had natural conditioned reflexes for pickled plum and orange but did not show any conditioned saliva for apple and biscuits. Some of the subjects responded to pickled plum, orange and apple, but not to biscuits. In general, the most abundant salivary response was to plum, and next to orange. What did these two orders of strength mean? We put two grams of each of these substances into the mouth, and unconditioned reflex saliva was measured. The result is, as in Table 2, that the strongest stimulus was pickled plum, and the weakest, biscuits. It must be clear that the natural conditioned reflexes of the subjects were preserved according to the strength of the substances to produce a real unconditioned reflex, and the weak stimulants to produce unconditioned ones : for instance, biscuits or apple could not maintain the conditioned reflex in a certain group of subjects.

TABLE 1. NATURAL CONDITIONED REFLEX IN JAPANESE ADULTS

Subjects	Sex	Age	Pickled plum	Orange	Apple	Biscuits
H.N.Y.	Female	27	24.5 mm	1.0 mm	0.0 mm	0.0 mm
O.N.Y.	Male	18	93.0 mm	2.0 mm	8.5 mm	8.0 mm
O.N.E.	Male	14	50.0 mm	12.0 mm	26.0 mm	11.0 mm

200 mm makes 1.0 ml.

TABLE 2. UNCONDITIONED REFLEX FOR PICKLED PLUM, ETC.

Substances	Subject No. 1	Subject No. 2
Pickled plum 2.0 g	1113 mm	900 mm
Orange 2.0 g	858 mm	330 mm
Apple 2.0 g	362 mm	64 mm
Biscuits 2.0 g	286 mm	65 mm
1/8 mol of tartaric acid solution 1.0 ml	449 mm	101 mm

200 mm makes 1.0 ml

NATURAL CONDITIONED REFLEX OF THE CHILD

In children, whole saliva was collected by absorbing it with tampons (Poth, 1938). The age of the children, boys and girls, was 9–11 years, all being primary school children. For them the best cotton was gossyium

absorbent of 400 mg divided into three parts, of which one was inserted under the tongue, two between the teeth and the inner side of each cheek. About every 3 min the cotton balls were taken out and at the same time new ones were inserted. The weight of the extracted cotton was measured all together, and saliva weight calculated.

Table 3 shows the resting whole saliva in grams for each 3 min period. It shows that the tampon must be a stimulus for resting salivation.

TABLE 3. RESTING WHOLE SALIVA IN CHILDREN

Name	Sex	Age	Resting saliva
Kihu	Male	9	3.01 g
Tami	Male	9	2.43 g
Tois	Female	9	3.84 g
Kako	Male	11	1.1 g
Hisu	Male	11	2.8 g
Etsi	Female	11	2.4 g
Emma	Female	11	1.5 g

Tampon method.

To test the natural conditioned saliva, a small chocolate candy which is familiar to the average Japanese child, an apple and a pickled plum were placed in front of the subjects. The results are shown in Table 4. All children showed conditioning for pickled plum and apple, and most did for chocolate.

TABLE 4. NATURAL CONDITIONED SALIVA IN CHILDREN FOR CHOCOLATE, APPLE AND PICKLED PLUM

Name	Sex	Age	Conditioned saliva (reduced by resting saliva) for		
			Chocolate	Apple	Pickled plum
Kihu	Male	11	0.22 g	0.54 g	0.80 g
Tami	Male	11	0.49 g	0.63 g	0.69 g
Yoyo	Female	11	0.0 g	0.17 g	1.05 g
Huna	Female	11	0.13 g	0.91 g	0.89 g

Tampon method.

EXTERNAL INDUCTION OF THE NATURALY CONDITIONED REFLEX IN MAN

The sight of a pickled plum makes the saliva flow in every Japanese, adults as well as children. This shows evidence of the existence of the

natural conditioned reflex in man. The amount of the reflex saliva for plum was almost constant. When the same sight of a plum is presented to the same subject in the form of another man eating it, does the amount of natural conditioned saliva change then? Does it produce an excitation or an inhibition?

Table 5 shows the parotid saliva when a plum was shown to the subject as well as when a plum was shown being eaten by the other man. In the former case, the saliva flowed 27 mm during 3 min, reduced by the resting saliva: but, in the latter case, it flowed 178 mm. The same holds for orange or apple, and is especially noticeable in the case of biscuits. As already stated most subjects had no trace of natural conditioned salivary reflex for biscuits, but when biscuits were shown being eaten by another man, conditioned saliva was produced, as shown in Table 5.

TABLE 5. EXTERNAL INDUCTION OF NATURAL CONDITIONED REFLEX IN JAPANESE ADULTS

Subjects	Sex	Age	Natural stimuli	Natural conditioned reflex	External induction of natural conditioned reflex
K.I.M.	Male	19	Damson plum	2.2 mm	20.2 mm
T.K.S.	Male	18	Damson plum	8.0 mm	80.5 mm
H.R.N.	Male	23	Pickled plum	50.0 mm	216.0 mm
T.K.S.	Male	18	Pickled plum	27.0 mm	178.0 mm

200 mm makes 1.0 ml.

A control experiment consisted of the second person eating paraffin wax of the same amount as a pickled plum; the saliva not appearing. We propose naming this phenomenon " external induction ", for the term " induction " was applied by Pavlov to the phenomena, necessarily accompanied without correspondent external stimuli in the excitation or the inhibition that occurred in the conditioned reflex.

EXTERNAL INDUCTION OF A NATURALLY CONDITIONED REFLEX IN CHILDREN

How does the external induction of a natural conditioned reflex work in children? Table 6 shows the whole saliva of a boy when shown a choco-late or plum. It also shows the result when these were eaten by the other boy or girl. In this case his natural conditioned saliva for chocolate was 0.15 g, for apple 0.35 g and for plum 0.42 g. External induction was 0.34 g for chocolate, 0.48 g for apple, 0.76 g for plum.

The external induction of a natural conditioned reflex is, in a proper sense, due to the physiological or to the combination of physiological and psychological mechanisms, which cannot be determined with certainty,

TABLE 6. EXTERNAL INDUCTION OF NATURAL CONDITIONED SALIVARY REFLEX FOR CHOCOLATE AND PLUM

Conditioned reflex for	Chocolate	Apple	Plum
Natural conditioned reflex	0.15 g	0.35 g	0.42 g
External induction	0.34 g	0.48 g	0.76 g

Kihu, male, 11 years old. Tampon method.

but it must be examined more precisely from the standpoint of nervous physiology. In other words, it needs further experiments with dogs as well as with man. The authors presume that it serves as an important clue for the study of some social behaviour of the conditioned reflex in the nervous system—i.e. from the standpoint of physiology.

The external induction was determined when a subject was alone as well as when he was in a group of several subjects watching the eater. Table 6 shows that external induction of 10 persons averaged 0.17 g when one person was eating chocolate, while, that of one person was -0.22 g when 10 persons were eating chocolate. The case of the pickled plum was not the same in principle, i.e. 10 persons averaged -0.11 g when one person

TABLE 7. EXTERNAL INDUCTION OF NATURAL CONDITIONED REFLEX OF CHILD, ALONE AND IN A GROUP OF CHILDREN

Unconditioned stimulus	Number of persons who eat	Number of persons who watch the eater	Natural conditioned saliva External induction	Difference
Chocolate	10	1	0.59 0.37	-0.22 g
	1	10	0.59 0.76	$+0.17$ g
Pickled plum	10	1	0.90 1.12	$+0.22$ g
	1	10	0.90 0.72	-0.18 g
Lunch	10	1	0.89 0.71	-0.18 g
	10	10	0.89 1.21	$+0.32$ g

was eating a plum.   And that of one person was +0.29 g when 10 persons were eating plums.   We had a similar case for the lunch.   It was −0.18 g when 10 persons were eating lunch, the same as in the case of chocolate. This time we determined that of 10 persons when another 10 persons were eating lunch.   The external conditioned reflex averaged +0.32 g.

What does the above mean ?   To interpret the quite unexpected results, we made a temporary hypothesis that in the case of a food which children were fond of, the external induction would be positive.   On the other hand, in the case of a food to which children had some antipathy, it would be negative—in other words, it would arouse inhibition.   In the case of a group, against a group, the external induction would be enhanced.   More details must await further investigation.

DISCUSSION AND SUMMARY

Parotid saliva can be measured accurately by a suction cup or the use of tampon.   In human beings (adults of both sexes aged 18–25 and children aged 9–11) we can detect the existence of natural conditioned reflex, in everyday food.   A weaker stimulant in unconditioned saliva, is not maintained, but as for a stronger one in unconditioned stimulation, a natural conditioned salivary reflex can be detected every time.

Another more noticeable fact is, that if one uses the natural conditioned stimulus, for instance, the sight of a pickled plum by a Japanese certainly arouses conditioned saliva, but the sight of a pickled plum eaten by some other man shows more abundant saliva.   This phenomenon is called for the time being " external induction " of the human natural conditioned reflex.

If the number of persons eating food was greater than the persons watching, the external induction increased in one ratio and decreased in another ratio, which would show interaction of people in a mass.

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OLFACTORY IDENTIFICATION OF CHEMICAL UNITS AND MIXTURES AND ITS ROLE IN BEHAVIOUR

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ELECTROPHYSIOLOGICAL investigation of olfactory and gustatory afferent fibres does not, in our present state of knowledge, allow us to analyse the discriminatory process itself. It is only behavioural data which actually reveal whether a particular animal confuses two sugars and distinguishes them from an acid ; or whether it has or has not the capacity to discriminate two molecules by smell, that is to say by their differential effects on the olfactory system. Thanks, however, to the work of Adrian, Pfaffman and of the Swedish and other workers, modern electrophysiological evidence has given us an idea of both the physicochemical and the intrinsic nervous mechanisms which, at different levels, account for the phenomena demonstrated by behavioural methods.

In this report on a very wide field of research, I shall limit myself to some considerations and hypotheses suggested by comparing electrophysiological and behavioural data about olfactory discrimination. Afterwards I shall briefly draw attention to the part played in animal behaviour by chemical analysis of the external world, using as an illustration some of my own recent work on learning by rats of specific differential appetites.

The division into four gustatory qualities is not exclusively the result of subjective analysis in humans. This analysis in the human subject, moreover, is less subjective than it seems, since it provides evidence through objective responses that, for instance, two sugars are confused and add their quantitative effects, that two acids are also confused and also add their effects, etc.

In many animal species, from invertebrates to man, behavioural methods have confirmed the same phenomena ; of discrimination or no discrimination, of additivity or no additivity: it can thus be found out that in aminals, also, the discriminative capacity of the gustatory system is limited to the separation of four groups of stimuli—four groups plus a single stimulus consisting of pure water, as we know through the elegant work of Zotterman and his colleagues. This functional characteristic of taste, permitting a rough analysis of the chemical medium in terms of four biochemical properties of these stimuli, is a fundamental one. It provides a

more useful differentiation of the gustatory from the olfactory apparatus than do anatomical and other functional criteria, and gives an exact definition of the former.

In olfaction, as in taste, chemical differentiation depends on a supply of information from the external environment. But in olfaction this analysis is carried out both quantitatively and qualitatively at the molecular level, quantitatively by the threshold activity of molecular units, qualitatively with regard to the possibility of identifying a single molecular unit. Apart from certain cases where confusion occurs, every active molecule is separated from others by a specific quality—its own odour. Olfaction is the sensation of chemical " individualization ", an improved definition first introduced by H. Piéron. Quantitatively, and even more so qualitatively, molecular analysis is the basis of the olfactory sense. At the same time this analysis of chemical units and mixtures is not, of course, perfect. The information supplied to the central nervous system by means of the olfactory pathway as to the chemical structure of the environment is subjected to a certain amount of distortion. Both in animals, whose discriminatory spectrum has been determined, and in the human being, on the basis of objective data, olfactory discrimination does not coincide with the strictly chemical differentiation. The sensory qualitative unit does not correspond exactly to the chemical units and mixtures. Where chemical units (definite atoms and molecules) are concerned, olfactory analysis sometimes falls short of the molecular level and sometimes goes beyond it. The first of these conditions applies in some cases where molecules of different chemical structure (e.g. nitrobenzene, benzaldehyde, the musks, etc.) are confused; as in taste, appearing to have the same quality. The second condition probably applies where, as shown in human olfactory discrimination, the smell of a given molecule may still be analysed in a mixture of different qualitative components (e.g. the smell of isoborneol perceived together with both camphor and musty odours).

The analysis of the kind of chemical mixtures which form most of the biological stimuli affecting behaviour (foods, body odours, etc.) suffers the same kind of distortion. Sometimes the mixture cannot be analysed olfactively. This is the case, in humans, for most natural products such as vegetable and animal odours. Smells like those of an essential oil, a vegetable oil, or the flavour of coffee, which are very complex chemical mixtures, appear as specific odours which cannot be analysed. I do not know of any experiment in animals which demonstrates that the response to a mixture is given as if to a single total stimulus ; but such a finding is highly probable and would be easy to confirm by adulteration of these mixtures. With some mixtures, on the other hand, both in humans and animals, an olfactory analysis of the components does occur. In humans this is generally the case with synthetic mixtures, such as commercial perfume.

For animals, I shall give below an example in which a flavour was discriminated by a rat when it was added to the familiar synthetic food.

In all such discrepancies between the olfactory and the chemical identity of molecular units and mixtures, it is very important to point out that discriminatory performance, in man, depends to a great extent on learning and previous experience and the subject's knowledge of the meaning of the stimuli concerned. The same is true in animals, where discriminatory performance also depends on learning, on experimental or physiological conditioning of the individual. A given chemical mixture, such as a food, which is not olfactively analysed by a novice, can be by an expert. Two molecules whose odour is confused by a novice will be distinguished olfactively by a chemist " knowing " them.

These commonplace facts indicate the origin of distortions of olfactory information, and of the variable and apparently capricious limits of olfactory discriminatory performance. The factors determining these distortions and limits are chiefly central in nature. The functioning by the olfactory apparatus is integrated, more than other systems are, in the regulatory schemes of the central nervous system. Modern neurophysiology has emphasized the very important role of centrifugal pathways acting at different levels of sensory systems to regulate and modulate their quantitative and qualitative responses. It is highly probable that these centrally regulated responses, depending on inherited connections and on the physiological role of olfactory stimuli in alimentary, sexual and other behaviour, play a fundamental role in olfaction and especially in qualitative discrimination of different stimuli. The peripheral mechanism for molecular differentiation, of course, forms the background for this and determines the ultimate limits of discriminatory capacity. But upon these messages coming up from the periphery—possibly even before arrival in the central nervous system—inhibitory and facilitatory processes obviously act, operating selectively within separate or mixed patterns, and analogous to those which have recently been shown to act at the retinal level in the visual discrimination of forms.

This analogy between the peripheral mechanisms for olfactory and for pastial visual discrimination—first suggested by Adrian in 1942—has also allowed an idea to be formed of the relation between peripheral and central mechanisms in discriminative performance as it appears in behaviour.

According to Adrian's well-known electrophysiological data, confirmed at the peripheral level by Ottoson, the specific message more or less characteristic of a given stimulating molecule is elicited by means of a pattern of differential activation by this given molecule of all or of specific numbers of functionally independent units. Thus, the action of a molecule on the olfactory surface would be the equivalent of the action of the image of an object on the retina, with its spatial and perhaps also its chromatic pattern.

On the basis of this analogy it seems possible to suggest the hypothesis that the overall mechanism of the discriminative process in olfaction acts in the same way as that involved in the visual discrimination of " anamorphous " figures.   In " anamorphous " or superposed figures, two figures known by the subject are mixed.   The perceptual phenomena involved in the separation or discrimination of these mixed figures are well known, especially the important role, in the discovery of the secondary figure masked in the predominant form, of the meaning of this figure and of the knowledge of this masked figure by the subject.   These perceptual phenomena are the exact parallel of those revealed in the olfactory discrimination of more or less different impulse-patterns induced at the peripheral level by two molecules, and the isolation of different specific patterns inside the overall patterns induced by a complex mixture of molecules, and are dependent on the same central factors associated with the " meaning " of the stimuli.

    To illustrate this assumption I shall give a single example.   Suppose that the pattern induced at the peripheral level by the molecule of hexachlorexane may be pictured as in A (Fig. 1), by the differential activation of only
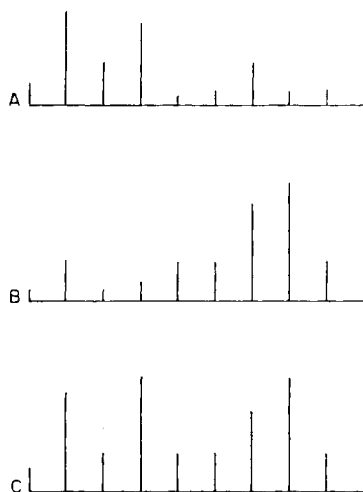

FIG. 1

ten independent units.   Suppose also that the pattern induced by camphor may be described as in B.   If the specific pattern of isoborneol is that illustrated in C, consisting of the superposition of the patterns A and B, one can explain why we smell the specific odour of isoborneol as a mixture of musty and camphor odour (as with a mixture of hexacyclohexane and camphor).   One can also explain why we differentiate these two qualities in the order of a molecular unit when we know, and only if we already know, the separate odours of musty and of camphor.

This same hypothesis and somewhat arbitrary examples might be used to explain all the above described phenomena of behavioural discrimination of chemical units or mixtures, and also many data about masking, and compensatory and reciprocal interactions of odours in mixtures. This explicative value justifies such a hypothesis. It may also be regarded as an indirect demonstration of the fecundity of Adrian's idea of a spatial-temporal pattern of impulses as a productivity basis for discrimination of odours. It may also serve as a reminder and as an illustration that the correlation of peripheral patterns of discharge with behavioural discriminatory performance in olfaction, as in taste, is somewhat hopeless ; and that therefor it may not be the proper basis for describing the discriminative process at this level so as to account for behavioural data. The study of more complex cortical and subcortical mechanisms which integrate and regulate the peripheral messages might one day make possible this correlation with behavioural data.

We are shortly coming to the role of olfactory chemical analysis in different patterns of behaviour of animals. The intervention of olfactory stimuli in behaviour, in preference to or in association with other sensory modalities (especially vision), corresponds to and is always explained by the ability to achieve by olfaction a biochemical analysis of the environment, a specific identification of a given molecule or of a given mixture of molecules. The sources of stimuli for animals are as follows : their respiratory medium, their food, their sexual partners, their associates and foes—that is to say the more fundamental elements concerned in their individual survival and the survival of the species.

In alimentary, sexual, social, warning and other behaviour where olfactory cues are concerned, the identification of specific chemical materials is always the basis for elementary reactions of attraction or repulsion, or for more elaborate behaviour. The identification of the odour of its associates in Phloxinus Levis (Göz, 1941), of the odour of its own young by the mother in the rat (Beach, 1956) ; the discrimination in many species of sexual odours (as I have shown in the rat : Le Magnen, 1952), the specific warning effect of L-serine on the salmon (Brett and co-workers, 1952–54) : all these are examples of the way in which olfactory stimuli and their accurate identification are used by animals in determining behaviour. The familiar behaviour of the dog able to follow an individual trail consisting of infinitesimal traces of specific bodily odours absorbed on the soil, is the simplest and best example of performances in which the olfactory molecular analyser alone is concerned.

In the field of my own work on regulation of food intake in the white rat, I believe I have shown an example of the role of discrimination of olfactory stimuli in the learning and manifestation by animals of specific appetite in accordance with their metabolic needs.

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The basic experiment is the following. Twenty rats, living in individual cages, receive daily two meals each lasting one hour, in which a constant alimentary mixture is offered. This alimentary synthetic mixture is offered in 2 forms, given alternatively in successive meals : the form A, consisting of the diet flavoured by addition of a trace of an odorant, citral ; and the form B, the same mixture flavoured by another odorant, eucalyptol. In a group of 10 rats free intake of the form A is immediately followed by a subcutaneous injection of a solution of glucose in an amount equivalent to 25 per cent of the caloric intake in the preceding meal. In the same animals, free intake of the other form of the food, flavoured with eucalyptol, is associated with a postprandial administration of subcutaneous saline. In the other group of 10 rats intake of the form A (citral) is followed by the administration of saline, and that of the form B (eucalyptol) is followed by the subcutaneous injection of the glucose solution. After 21 days of this treatment it was found that in a choice between the two forms the rat exhibits a differential appetite. At that time the addition of citral to the food for the animals of the first group, and of eucalyptol in the second group, in a choice as well as in individually presented meals, reduced the intake of the meal by 25 to 30 per cent. Thus at this time, differentiation of the two flavours is the only basis for the specific appetite induced in a quantitative manner by the conditioning postingestive action of glucose during the preceding period.

It is highly probable that physiologically also it is on the basis of the natural flavours of foodstuffs and by accurate olfactory discrimination of these flavours, that the animal learns its specific appetite, the refusal of non-alimentary or toxic materials, and the acceptance of different foods in accordance with their caloric and specific properties and in accordance with metabolic needs. Concerning the role of olfactory cues, another series of experiments has shown the particular and privileged role played by olfactory discrimination in this alimentary learning. The general procedure in this series is the same as described above, except that the postin-gestive conditioning factor is not glucose, but the addition to the mixture of a small dose of D-amphetamine. This constant factor is in action in 5 groups of 20 rats each. In the first, an olfactory differentiation is established between two forms of the mixture as before—the one associated with the presence of amphetamine, the other not. In the second group a differentiation of texture is offered to the rat by a difference in the granu-losity of the sugar contained in the mixture. In the third group a difference of luminosity (black-white) between the two forms is brought about by addition to one of them of a black colorant. In the fourth group constant position in the cage affords a possibility of discrimination of the two forms by many complex spatial cues. The fifth group is a control in which no sources of discrimination between the two forms are made available. Here

the pattern of alternation of meals with and without amphetamine is the same, but with the mixture unchanged and constant, and in the final choice the same food (with any further addition of amphetamine as in the other groups) is offered in the two cups.
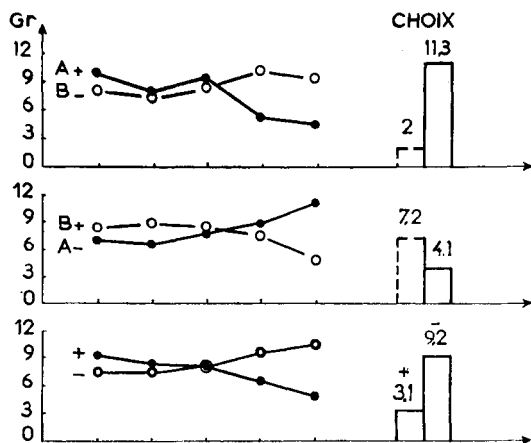

FIG. 2. Group I : Olfactory discrimination. Each point represents the average of two meals on two days in succession (during the conditioning period).
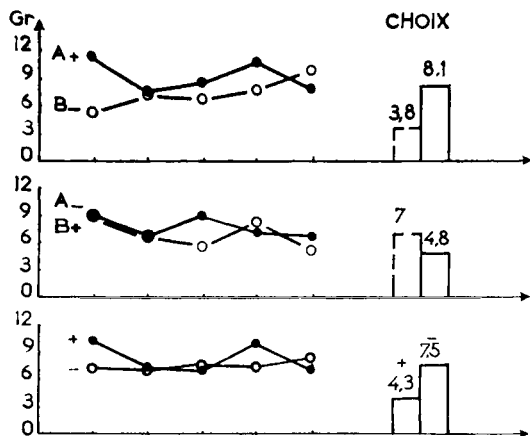

FIG. 3. Group II : Tactile discrimination.

In Figs. 2 to 6, it is shown that the differential appetite at the end of the leaning period is evident and various with the different sensory cues offered to the rat.   Differential appetite is maximal with olfactory discrimination, with a ratio of 3 : 1 in the intake of the two forms in the choice, compared
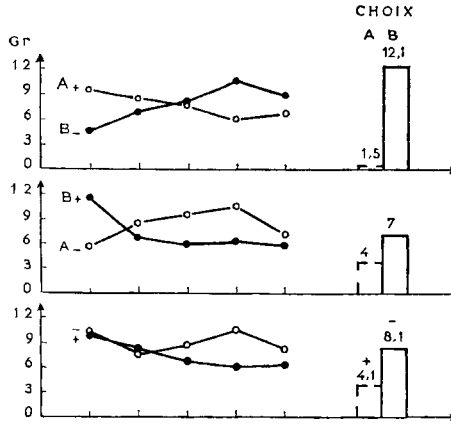

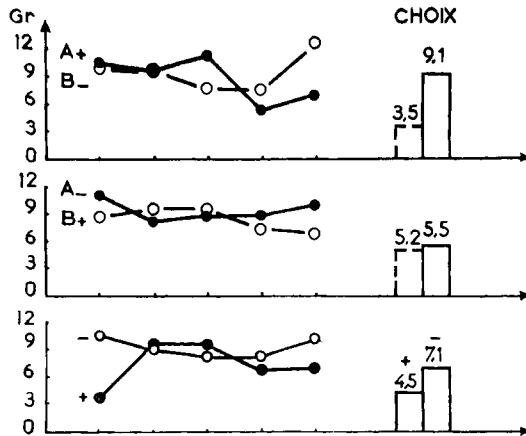FIG. 4.   Group III : Visual discrimination.

FIG. 5.   Group IV : Spatial discrimination.

with 2 : 1 or less for the other cues (tactile and visual discrimination). In the absence of any source of discrimination at the oral level in the latter group, no differential appetite is learned, and in the final choice the rat does not exhibit any preference.
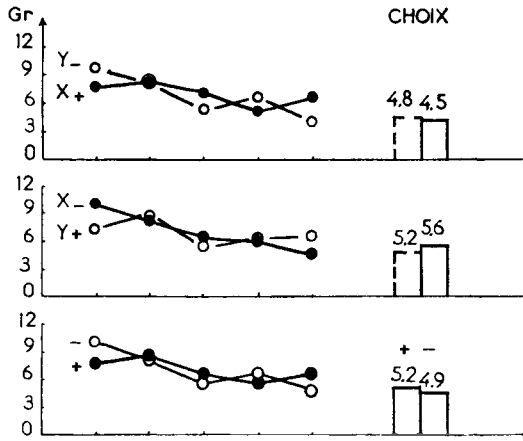
FIG. 6.   Group V : No discrimination.

Thus, it is shown that olfactory stimuli and olfactory discriminations of chemical units and mixtures, in rats, as in humans with their more capricious appetites, are the best source of learning of appetites in accordance with different properties of the foodstuff and metabolic bodily needs.

THE ROLE OF TASTE AND SMELL
IN THE REGULATION OF FOOD
AND WATER INTAKE

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It is commonly assumed that taste and smell play little or no role in the long-term quantitative regulation of food and water intake. This view is based on a variety of different kinds of evidence.

First, animals regulate their caloric intake over a wide range of dietary adulterations. If the nutrient content of a solid powdered diet is decreased by mixing it with cellulose (Teitelbaum, 1955) or with kaolin (Adolph, 1947 ; Kennedy, 1950) up to as much as 75 per cent, the animals maintain appreciable intake. It is only when the bulk of the diet becomes tremendous that the animal will fail to eat enough to survive (Smith, Pool and Weinberg, 1962). If water is added to a liquid diet, normal intake is maintained when as little as 2 per cent nutrient is present (Adolph, 1947). Normal animals will thus face extreme overhydration to maintain their caloric intake.

Second, direct manipulation of the taste of the diet seems not to affect caloric intake at all. If food is sweetened by mixing it with dextrose (Teitelbaum, 1955) caloric regulation is maintained at the same level. If the diet is made quite bitter by mixing it with up to 1.25 per cent quinine hydrochloride, rats continue to regulate caloric intake (Teitelbaum and Epstein, 1962). For water intake, the picture is quite similar. When quinine hydrochloride is added to their water, rats continue to drink it up to a concentration of 1 per cent (Teitelbaum and Epstein, 1962). This is close to the maximum amount of quinine that will dissolve in water and it is the point at which the fluid becomes lethal if ingested in normal quantity. Therefore, the animals continue to drink quinine solutions until they become actually poisonous.

Third, regulation continues in the absence of taste and smell. Several attempts have been made to achieve complete surgical denervation of taste and to study the regulation of food and water intake in its absence. In dogs, Bellows and Van Wagenen (1939) sectioned the fifth cranial nerve to denervate the buccal cavity. In other dogs, they cut the chorda tympani

and glossopharyngeal nerves to diminish the sense of taste, and also removed the olfactory bulbs to eliminate smell. They showed that none of these removals had any effect on thirst or the regulation of water intake. Since they mentioned no feeding aberrations, we may assume that the regulation of food intake was likewise undisturbed.

However, as Richter (1956) has pointed out, it is extremely difficult to achieve complete surgical denervation of taste. In the rat, he found taste buds present even after combined section of the chorda tympani, glossopharyngeal, and the pharyngeal branch of the vagus. Pfaffmann (1952) has shown that only partial loss of taste results from combined removal of the chorda tympani and glossopharyngeal nerves. When the pharyngeal branch of the vagus is also removed, severe impairment of chewing and swallowing can result, with complications arising from the aspiration of food during feeding. Our own attempts at removals have verified Pfaffmann's findings. Other workers have attempted to destroy the sense of taste by ablations in the central nervous system. Cortical removal of the projection areas yield only a partial loss of taste sensitivity with considerable recovery of function (Benjamin and Pfaffmann, 1955 ; Bagshaw and Pribram, 1953), and even by destroying the thalamic nuclei projecting to the taste areas, it is difficult to completely remove the sense of taste (Ables and Benjamin, 1960 ; Oakley and Pfaffmann, 1962 ; Andersson and Jewell, 1957).

We have, therefore, turned to an alternate method of removing the influence of taste and smell from the regulation of food and water intake. Epstein (1960) has devised a permanently implanted gastric tube for the rat that does not require the use of gastrointestinal surgery and does not interfere with the animal's normal feeding and drinking. The chronic stomach tube is shown in Fig. 1.

A slender polyethylene tube is slipped into the rat's nostril, through the nasopharynx, into the esophagus, and down into the stomach. The outer end of the tube is brought under the skin of the snout and scalp to the top of the skull where it is anchored firmly and permanently by screws and dental cement. Water or liquid food can now be pumped directly into the animal's stomach, thus completely by-passing the nasal and oro-pharyngeal receptors for taste and smell. By training the rat to press a lever to inject water or a liquid diet directly into its own stomach, we can study the regulation of food and water intake in the absence of all oro-pharyngeal sensations, particularly taste and smell, and without even the consummatory acts of chewing or swallowing. This arrangement is shown in Fig. 2.

The rat is first taught to press a bar for the delivery of fluid into a cup in its cage. It ingests the fluid by mouth. Then the pump is connected to the animal's gastric tube so that when the animal presses the bar, it delivers food or water directly into its own stomach. A watertight swivel joint
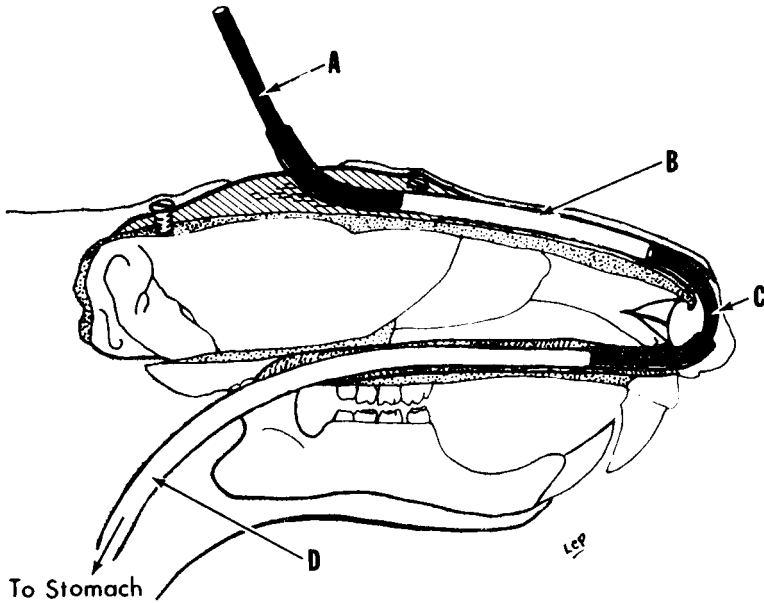
FIG. 1. The course of the nasopharyngeal gastric tube shown in a schematic drawing of a midsagittal section of the rat's head. (From Epstein and Teitelbaum, 1962b.)
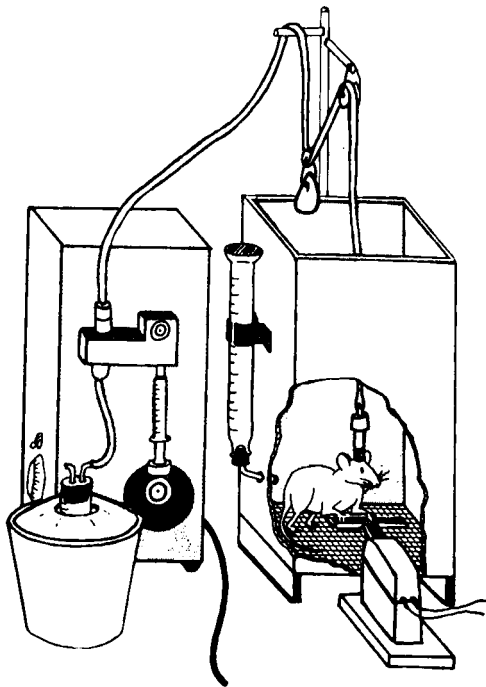

FIG. 2. Schematic drawing of the apparatus for intragastric self-injection by the rat. (From Epstein and Teitelbaum, 1962b.)

(Epstein and Teitelbaum, 1962) coupled between the animal's gastric tube and the pump allows the animal to spend day and night in the cage. The joint swivels with the animal and prevents its movement around the cage from kinking the delivery tube. The rat can therefore feed or water itself day and night without tasting, smelling, or feeling food or water in its mouth, and without even the act of eating or drinking. Does regulation of food and water intake continue in the absence of taste and smell? Let us first consider water intake.

As shown in Fig. 3, from the work by Epstein (1960), the rat will regulate its daily water intake, and even compensate for the increased water loss of diabetes insipidus by pressing a bar for direct injection into its stomach.
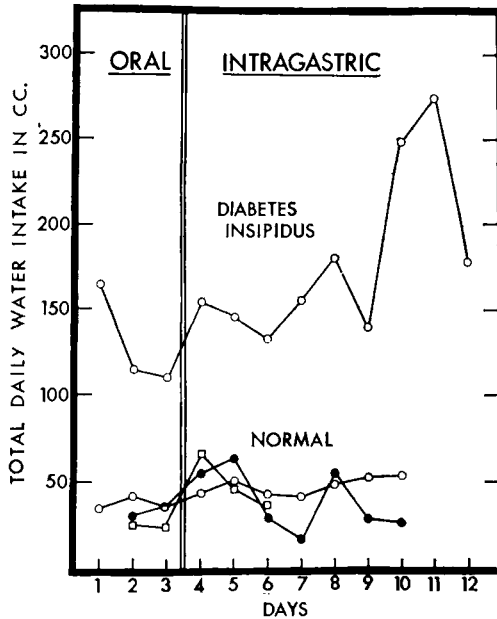
FIG. 3.   Oral and intragastric water intake in normal rats and in an animal with diabetes insipidus. (From Epstein, 1960.)

Food intake is also regulated normally in the absence of taste and smell. Epstein and Teitelbaum (1962) have shown that rats feeding themselves by intragastric self-injection regulated their daily food intakes and body weights with remarkable precision for periods of 13 to 44 days despite variations in the concentration of the diet, the size of individual stomach loads and the number of presses required for a single load. All of the animals continued their normal slow weight gain during the period of intragastric feeding. Figure 4 shows 3 days of oral feeding and the data of the first 25 days of the intragastric period of the animal studied for the

longest time. It includes records daily of body weight, food intake, number of self loads, and total number of responses (bar presses).

Note first that the transition from oral to intragastric ingestion is made with ease. Two indices of regulation were used. First, decreasing the concentration of the diet to 50 per cent by dilution with water resulted in a prompt and sustained doubling of intake, number of self loads, and number of responses. The adjustment downward when the diet was returned to full strength, was equally precise.
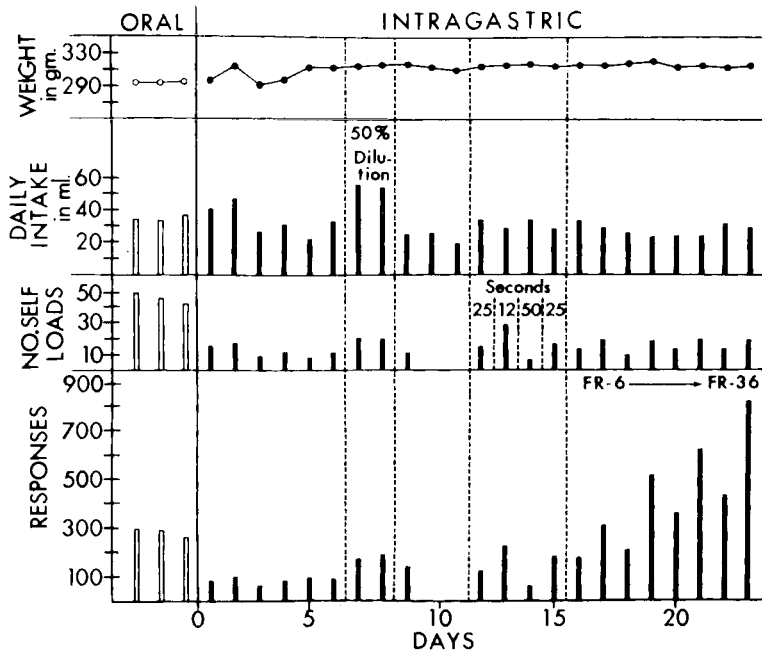

FIG. 4. Body weight, daily food intake, the number of self-loads, and the number of daily responses during 3 days of oral food intake and 25 days of intragastric intake in the normal rat. (From Epstein and Teitelbaum, 1962b.)

Second, food intake and body weight were regulated with comparable precision in the face of sudden halving or doubling of the size of the individual stomach loads by appropriate and precise changes in the number of loads taken. While working for a standard load of 2.5 ml on the day before these changes, the animal in Fig. 4 obtained 34 ml of diet by injecting itself 15 times. When the size of the load was halved the animal obtained 28 ml in 28 injections, and when the load was doubled the animal obtained 33 ml in only 7 injections. In addition, motivation for food was maintained. Total daily responses rose proportionally as the number of bar presses required to obtain each load was gradually increased from 6 to 36 so that intake and body weight remained well within normal limits. When

the usual 6 bar presses were required, (the 15th day in Fig. 4) the animal obtained 28 ml of liquid diet by pressing 180 times for 17 self loads. One week later, when 36 bar presses were needed for each gastric load, the animal pressed 822 times to obtain 18 self loads and 29 ml of liquid diet.

Although the method of intragastric feeding by self-injection appears to eliminate the influence of taste and smell from the regulation of food and water intake, it is conceivable that other possibilities of tasting the diet might have been present. Thus, taste might have resulted from some slight regurgitation of the diet after each injection. Or perhaps some intravenous taste might arise from some nutrients after absorption from the stomach. To control for this possibility, we allowed three rats to feed themselves intragastrically for three days with a diet adulterated with 0.05 per cent quinine hydrochloride (5.0 ml of a 1.0 per cent stock solution added to 95 ml pure diet). This adulterated diet is so bitter that animals ingesting it by mouth either refuse it completely or decrease their daily intake very markedly. Figure 5 shows the result of such an experiment.

The average daily intake of the pure liquid diet of both groups of animals

	Pure diet	0.05% quinine Adulterated diet		
		Day 1	Day 2	Day 3
Oral	42	13	24	36
Intragastric	29	33	33	30

Fig. 5. Daily food intake (in ml.) during one day on pure liquid diet and three succeeding days on 0.05 per cent quinine adulterated diet of a group of rats feeding orally ($N = 4$) and a group feeding intragastrically ($N = 3$). (From Epstein and Teitelbaum, 1962b.)

is shown for one day before adulteration and for three days of quinine adulteration. Oral intake of the quinine adulterated food by the control group fell sharply on the first day in all four animals and then rose to slightly below normal on the third day of the test. Averaged across the 3-day period, oral intake of the adulterated food was only 57 per cent of the previous intake of the pure liquid diet. This was reflected in an average loss in body weight of 12 g suffered by the animals in the same period. In contrast, the intake of the animals ingesting the adulterated diet by intragastric self-injection was essentially unchanged and they lost no weight during the quinine test period.

The rats studied here fed themselves normal amounts of food and regulated their body weights by injecting a liquid diet into their own stomachs on an *ad libitum* schedule for as long as 44 days. During all of this time,

the food did not pass through the mouth or pharynx. And at the end of this period, intake was not depressed by quinine adulteration. Taken together, these two facts make it virtually certain that the food did not stimulate any of the receptors in the olfactory mucosa or the oral pharynx during the act of ingestion.

Clearly, the central neural mechanism controlling food intake can operate effectively without receiving sensory information about the taste and smell of the food being ingested, or its feel in the mouth, and without proprioceptive feedback from the muscles involved in chewing and swallowing. Metering by mouth provided by the act of eating is not necessary for the regulation of food intake in the normal rat. Post-ingestion factors such as sensations from the gut and chemical or thermal changes in the blood reaching the central nervous system must be sufficient to control the onset of feeding, the size of individual meals, the total amount of food eaten during a single day and for longer periods of time up to more than a month. Since drinking has previously been shown to be similarly independent of oro-pharyngeal and olfactory sensations (Epstein, 1960), these considerations apply to drinking as well as feeding.

All of the above applies to the caged animal. Clearly, sensations from the mouth, olfactory mucosa and pharynx are of great importance for the management of consummatory behavior in the wild, where the animal must detect food in the environment, discriminate between the edible and inedible, and reject poisons. Specific hungers, such as the increased intake of salt that follows adrenalectomy also depend upon the animal's capacity to taste (Richter, 1956). What we are emphasizing is that although oro-pharyngeal sensations *are* essential when the animal must find food and identify it, they are not essential when the animal's only problem is how much to eat.

THE ROLE OF TASTE AND SMELL IN THE REGULATION OF FOOD INTAKE IN ANIMALS WITH HYPOTHALAMIC DAMAGE

But all these conclusions about the role of taste and smell in the regulation of food and water intake are based on the study of normal animals. And every study of regulation assumes normal motivation for food and water. It is taken for granted that if an animal does not eat, or does not regulate adequately, it is because regulation is impaired. We shall now attempt to show that regulation depends critically on the existence of a powerful urge to eat or drink. In normal animals, this urge overcomes all barriers to ingestion and makes taste appear trivial. But regulation cannot exist without adequate motivation. In brain-damaged animals, where motivation is impaired, taste becomes critical for regulation. We will show that taste and smell are powerful motivating stimuli—psychic energizers—that

provide the additional motivation necessary for regulation.   In these animals, the slightest change in the taste of food or water may mean the difference between life or death.

   There are two kinds of disturbances in feeding that result from hypothalamic damage.   Overeating results from lesions in the vicinity of the ventromedial nuclei.   Starvation occurs after damage to the lateral hypothalamic areas.   Let us first consider hypothalamic hyperphagia.   There appears to be no metabolic disturbance in animals with ventromedial hypothalamic lesions—they get fat because they eat too much (Brobeck, Tepperman and Long, 1943).   There are two phases in hypothalamic hyperphagia : an initial dynamic phase, immediately following the operation, in which the animal eats two to three times as much as normal, and gains weight rapidly.   Then after the animal has become obese, a static phase ensues, in which the weight levels off at a high plateau and the animal's food intake drops back to only slightly more than normal.   How well do such animals regulate their food intake in the face of changes in the quality of the diet?   Figure 6 shows the results of an experiment in which
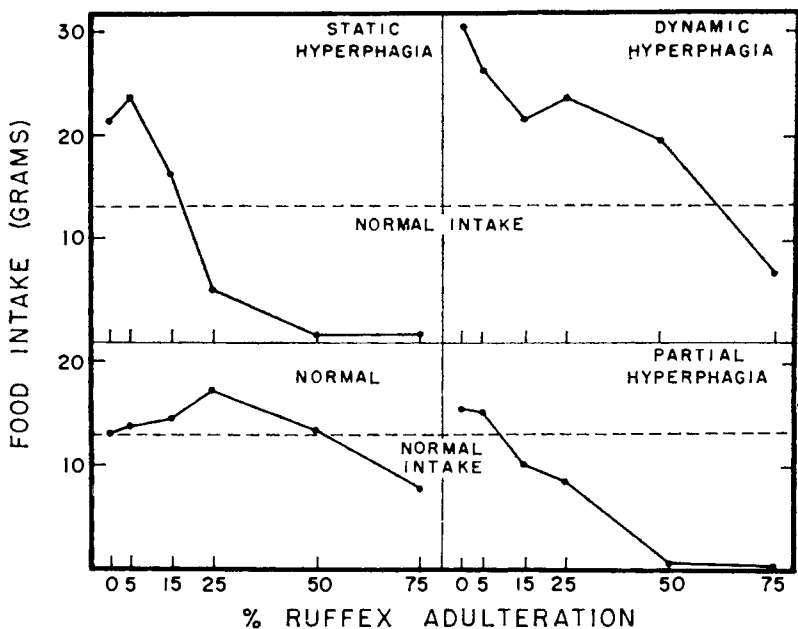

FIG. 6.   Food intake of normal and hyperphagic animals when offered a diet
mixed with non-nutritive cellulose.   (From Teitelbaum, 1955.)

the diet was adulterated with non-nutritive bulk (Teitelbaum, 1955). As described previously, normal animals regulate their intake when almost half the diet is non-nutritive cellulose, and eat appreciable amounts even

when 75 per cent of the diet is cellulose. In contrast, hyperphagic animals do not increase their intake when the diet is mixed with cellulose. Although they eat two to three times as much of the ordinary diet, they refuse to eat the cellulose-adulterated diet when it contains as little as 25 per cent cellulose. The effect is particularly marked in obese hyperphagic animals, which eat little or nothing for a week at a time and lose a great deal of weight.

If as little as one part of quinine sulphate by weight is mixed in 800 parts of their ordinary food, as shown in Table 1, obese hyperphagics stop eating although normal animals are unaffected (Teitelbaum, 1955). If the diet is sweetened with dextrose, as shown in Table 2, normal animals regulate

TABLE 1. THE EFFECT OF 0.125 PER CENT QUININE SULPHATE ON THE FOOD INTAKE OF NORMAL, DYNAMIC, AND OBESE HYPERPHAGIC RATS.   EACH NUMBER REPRESENTS MEAN DAILY INTAKE FOR 5 DAYS. (FROM TEITELBAUM, 1955.)

Group	N	Standard diet	0.125% quinine	P
Normal	7	16.1	15.8	—
Obese	7	19.5	2.1	<0.01
Dynamic	5	25.4	24.4	—

TABLE 2. THE EFFECT OF FLAVOURING THE DIET WITH DEXTROSE ON THE FOOD INTAKE OF NORMAL, DYNAMIC, AND OBESE HYPERPHAGIC RATS. EACH NUMBER REPRESENTS MEAN DAILY INTAKE FOR 5 DAYS. (FROM TEITELBAUM, 1955.)

Group	N	Standard diet	50% dextrose	P
Normal	7	16.9	14.2	<0.01
Obese	5	22.2	29.3	<0.01
Dynamic	5	26.5	26.5	—

their intake by eating slightly less, whereas obese hyperphagics show marked increase in food intake (Teitelbaum, 1955). All these effects are most exaggerated in obese hyperphagic animals, but Graff and Stellar (1962) in a recent analysis of the effects of positive and negative taste stimuli on feeding, have shown that all hyperphagics, obese or dynamic, react exaggeratedly to taste stimuli in their food. This finickiness shown by hyperphagic animals indicates a basic disturbance in their motivation for food, as was pointed out by Miller, Bailey and Stevenson (1950).

When such animals are required to feed themselves intragastrically, they again show impaired motivation coupled with an increased dependence upon taste and smell. Figure 7 shows the results of an experiment (done in collaboration with Mr. Dennis McGinty) in which a dynamic hyperphagic

animal fed itself intragastrically for 41 days   It can be seen that hyperphagic animals continue to eat and become obese when they feed themselves intragastrically.   They can respond appropriately to dietary dilution of the diet and will regulate intake by adjusting to the size of the individual
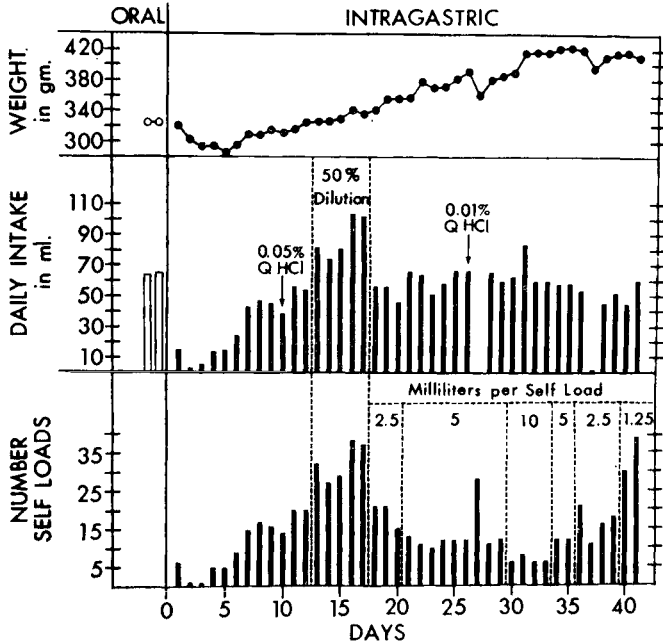

FIG. 7.   Body weight, daily food intake, the number of self-loads, and the number of daily responses during 2 days of oral food intake and 41 days of intragastric intake in the dynamic hyperphagic rat.

stomach load.   But in contrast with normal animals, they are quite sluggish in demonstrating such intragastric regulation.   Note that after the transition from oral to intragastric feeding, it took 7 days before the animal was willing to work hard enough to overeat intragastrically.   From the 1st to the 6th day of intragastric feeding, the animal's voluntary intake was markedly depressed.   Normal animals typically make the transition within the very first day of intragastric feeding.   The inertia shown by hyperphagic animals can be attributed to their impaired motivation for food and reveals the importance of taste in maintaining adequate regulation of their intake.

When an animal is switched from oral feeding to intragastric feeding, taste and smell are removed as a reinforcement from the diet.   Normal animals are sufficiently motivated to overcome the decrease in reinforcement and therefore they continue to regulate their intake without lag when feeding themselves intragastrically.   Hyperphagic animals, however, with

their impaired motivation, react exaggeratedly to the removal of taste and smell from the diet. They do not press the bar frequently enough to obtain sufficient food. It is only after they have had a long period of experience with the diet that they will work for it and demonstrate adequate regulation when they are fed intragastrically. This can be very clearly shown by providing the taste of food in addition to its injection into the stomach. If a tiny amount of food is injected into their food cup while they receive their full amount of intragastric injection, hyperphagic animals that are demonstrating this motivational inertia when first beginning to work for food will immediately display vigorous hyperphagia. They will work hard for food only if they get a slight taste of the diet in addition to the intragastric injection. They regulate precisely, work vigorously, overeat and gain weight rapidly. If the taste is removed again, however, they immediately relapse into a state of sluggish motivation. Therefore, the regulation that they could display is concealed because they are not adequately motivated. This shows clearly that taste acts as a powerful motivating stimulus to enable animals with impaired motivation to regulate normally.

THE ROLE OF TASTE IN THE FOOD INTAKE OF LATERAL HYPOTHALAMIC ANIMALS

Lateral hypothalamic lesions result in a syndrome of aphagia and adipsia leading to death (Anand and Brobeck, 1951). However, if these animals are kept alive by maintaining them with artificial tube feeding, they will eventually recover (Teitelbaum and Stellar 1954), and their recovery yields a great deal of information about the nature of the deficits induced by the lesions. Teitelbaum and Epstein in a recent analysis (1962) have shown that the lateral hypothalamic syndrome results from a combination of deficits in both feeding and drinking from which animals recover at different rates. There are four clear-cut stages in this recovery. As shown in Fig. 8, first we see the stage of aphagia and adipsia : the animal neither eats nor drinks anything and will die if not maintained artificially. Then follows a stage of anorexia and adipsia in which the animal can be induced to take highly palatable wet foods, such as wet cookies, chocolate, or a liquid diet. It ingests appreciable quantities but still does not eat enough to maintain its body weight. It still has to be tube-fed. It is as though it is drawn to the palatable diet by appetite, not driven to it by hunger. Taste provides sufficient motivation to induce the animals to eat but the urge to eat has not sufficiently recovered to the point where normal regulation can be demonstrated. However, the urge to eat does return. This is seen clearly in Stage III. The animals regulate their intake of the liquid diet and maintain their weight ; however, they are still adipsic. In this stage, such animals can be trained to feed themselves

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intragastrically. They regulate their intragastric intake of a diluted liquid diet normally and maintain their weight. Therefore, they no longer need the powerful motivating stimulus provided by taste to demonstrate adequate regulation of food intake. They have recovered sufficiently to regulate their

	Stage I ADIPSIA, APHAGIA	Stage II ADIPSIA, ANOREXIA	Stage III ADIPSIA, DEHYDRATION- APHAGIA	Stage IV RECOVERY
EATS WET PALATABLE FOODS	NO	YES	YES	YES
REGULATES FOOD INTAKE & BODY WT. ON WET PALATABLE FOODS.	NO	NO	YES	YES
EATS DRY FOODS (IF HYDRATED)	NO	NO	YES	YES
DRINKS WATER. SURVIVES ON DRY FOOD AND WATER.	NO	NO	NO	YES

FIG. 8. Stages of recovery seen in the lateral hypothalamic syndrome. The critical behavioural events are listed on the left. (From Teitelbaum and Epstein, 1962.)

caloric intake without the motivation provided by a highly palatable taste. But they still refuse to drink water. If offered only dry food and water, even after they have regulated their intake of a liquid food perfectly well, they refuse to drink, and become dehydrated. They therefore refuse to eat, and once again will starve to death. This stage is one in which they do not eat because they do not drink, and we have therefore called it the stage of adipsia with secondary dehydration aphagia. Many animals after lateral hypothalamic lesions show permanent adipsia. They have recovered the urge to eat but do not appear to possess true thirst. However they can be induced to drink fluids merely by making them taste sweet. They can be weaned gradually from the liquid diet by mixing it with more and more 10 per cent sucrose. From this in turn, they can be weaned to a 0.5 per cent or a 0.2 per cent saccharine solution. They ingest this fluid and therefore hydrate themselves sufficiently to eat dry food. At this point, they are taking fluid solely on the basis of its sweet taste. They have been tricked into hydrating themselves by offering them a sweet fluid, to which they respond as though it were a food. This has been demonstrated by showing that they increase their intake of saccharine solutions tremendously when they are deprived of food but they do not ingest it when made thirsty by complete deprivation of fluid or by dehydration with intraperitoneal hypertonic saline. Normal animals respond to saccharine either as a

fluid or as a food depending upon whether they are thirsty or hungry. Lateral hypothalamic animals seem to lack the capacity to detect true thirst. They eat the saccharine ; they do not drink it.

Finally, a fourth stage ensues in the recovery from lateral hypothalamic lesions. Most animals with small lateral hypothalamic lesions eventually begin to drink water and are able to eat sufficient food to maintain themselves. They appear, therefore, to be normally recovered. However, these animals still show impaired motivation for food and water. Adding a slight amount of quinine to their water, as little as 0.005 per cent is sufficient to cause them to refuse it completely day after day, become dehydrated, stop eating, and die. Normal animals, as mentioned earlier, will take 200 times as much quinine, up to 1 per cent, which is poisonous. In the same way, adding a slight amount of quinine to their food will cause lateral hypothalamic recovered animals to reject it entirely and to starve. Normal animals overcome the barrier to ingestion imposed by a strong negative taste and continue to eat and regulate normally.

Misleading interpretations of the lateral hypothalamic syndrome have resulted when investigators have offered these rats ordinary food and assumed that because they do not eat that they cannot eat. In these situations, death is an artifact—an artifact of the narrow range of acceptability demonstrated by lateral hypothalamic animals. Normal animals accept a wide range of foods from aversive to highly palatable substances. Lateral hypothalamic animals do not. Early in recovery they will completely refuse even ordinary foods such as purine powder or pellets and will die. But had they been offered more palatable foods, such as liquid diet, milk chocolate or wet cookies, they would have eaten, and, if more advanced in recovery, even regulated their intake perfectly. When sufficient motivation has been regained, taste and smell become less essential for intake. The animal will feed itself intragastrically without taste or smell, but by making the food or water a little less palatable, it can be thrown back into what appears to be the initial stages of aphagia and adipsia. Therefore, they still do not have the full vigorous urge to eat, and in fact, they may never completely recover it.

On the basis of these results with animals with hypothalamic damage, we may assign to taste and smell their rightful roles in the quantitative regulation of food intake. Normal regulation depends on adequate motivation. Taste and smell are powerful motivating stimuli. They are psychic energizers that contribute to the animal's hunger drive. However, in the normal animal, retaining intact all other sources of the urge to eat, they are dispensable. But when the drive is diminished by central neural damage, the importance of taste and smell is magnified, and they may become the dominant motives to eat. Therefore, whenever motivation is impaired, taste and smell are essential for regulation.

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THE RELATIONSHIP BETWEEN BODY
TEMPERATURE AND FOOD AND WATER INTAKE

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TWENTY-FIVE years ago Beattie (1938) stated : " The physiology of the hypothalamus is the physiology of the internal environment." This statement may have been an exaggeration. Nevertheless, numerous mechanisms of importance for the maintenance of a constant internal environment are undoubtedly controlled or influenced by the hypothalamus. This certainly holds true for the regulation of body temperature and the control of food and water intake. These hypothalamic functions have for some time been the subject of study in this laboratory, where unanesthetized goats have been used as experimental animals. Recently special attention has been paid to the thermoregulatory and alimentary effects of hypothalamic cooling and warming. The technique used has allowed local cooling or warming of the preoptic region and the anterior hypothalamus for long periods of time in unrestrained goats maintained in their normal environment (Andersson and Larsson, 1961 ; Andersson, Gale and Sundsten, 1962a ; Andersson, Ekman, Gale and Sundsten 1962c).

In the following an attempt will be made to review the results of these studies in the light of present knowledge of the regulation of body temperature and of mechanisms controlling food and water intake.

HYPOTHALAMIC TEMPERATURE AND THERMOREGULATION

The mechanisms maintaining a constant body temperature in warm-blooded animals have for more than a century received great interest and have become the subject of numerous experimental studies. The results of these studies have led to the present view that both peripheral temperature receptors and central temperature " detectors " participate in the regulation of body temperature. (For recent reviews see von Euler, 1961 and Hardy, 1961.) An abundant inflow from peripheral cold receptors may thus activate cold defence mechanisms such as shivering and peripheral

vasoconstriction. A raised body temperature, on the other hand, may cause the mobilization of various heat loss mechanisms (sweating, peripheral vasodilatation and polypneic panting) by the mediation of central " warm detectors " in the preoptic region and the rostral hypothalamus (the " heat loss centre ") (Magoun, Harrison, Brobeck and Ranson, 1938 ; C. von Euler, 1950).

The question still remains open whether parallel to central " warm detectors " there may exist central " cold ", or, rather, " hypothermia detectors " which serve to activate various cold defence mechanisms. Local cooling of the rostral hypothalamus in the dog generally induces shivering in spite of little or no inflow from peripheral cold receptors (Hammel, Hardy and Fusco, 1960). For this reason Hammel *et al.* (1960) have suggested that the rostral hypothalamus and preoptic region may be the site not only of central " warm detectors " with the task to prevent hyperthermia, but also the site of central " hypothermia detectors " having the opposite thermoregulatory function. The preoptic region and the rostral hypothalamus would then be regarded not simply as a " heat loss centre " but rather as a thermoregulatory " centre " of a more general character. Microelectrode studies of neuronal activity in this part of the brain do not support this view. The activity of numerous neurons are found to increase in response to a raised hypothalamic temperature, but no neurons seem to react specifically to a lowering of the temperature (Nakayama and Hardy, 1962). Further, it does not seem necessary to postulate the existence of central " hypothermia detectors " to explain the thermoregulatory effects of local cooling of the preoptic region and rostral hypothalamus of the goat (Andersen, Andersson and Gale, 1962). In this species even profound cooling of the rostral hypothalamus elicits shivering only if there is an inflow from peripheral cold receptors of if the shivering mechanism is facilitated by certain humoral or emotional factors. Thus in the calm goat, fully accustomed to the experimental conditions, local cooling of the preoptic region and the rostral hypothalamus induces shivering in a cold environment only (Fig. 1), or during local stimulation of peripheral cold receptors. But if these goats are subjected to mild stress or to an infusion of adrenal catecholamines, cooling of the " heat loss centre " may elicit shivering in a thermally neutral or even warm environment (Fig. 2). It thus seems as if the central " warm detectors " of the " heat loss centre " serve not only to activate various heat loss mechanisms but also as a brake on the shivering mechanism. However, only when there is a simultaneous, stimulatory " drive " on the shivering mechanism from peripheral cold receptors or from other sources (emotional excitation, sympathico-adrenal activation, pyrogens etc.) does an inactivation of these " warm detectors " by cooling elicit shivering. (For tentative scheme see Fig. 3).

It is well known that not only nervous, but also endocrine factors are of

importance for the maintenance of a constant body temperature.   The
contribution of adrenal and thyroid hormones to the metabolic response to
cold has been demonstrated in several ways.   Cold exposure is, for example,
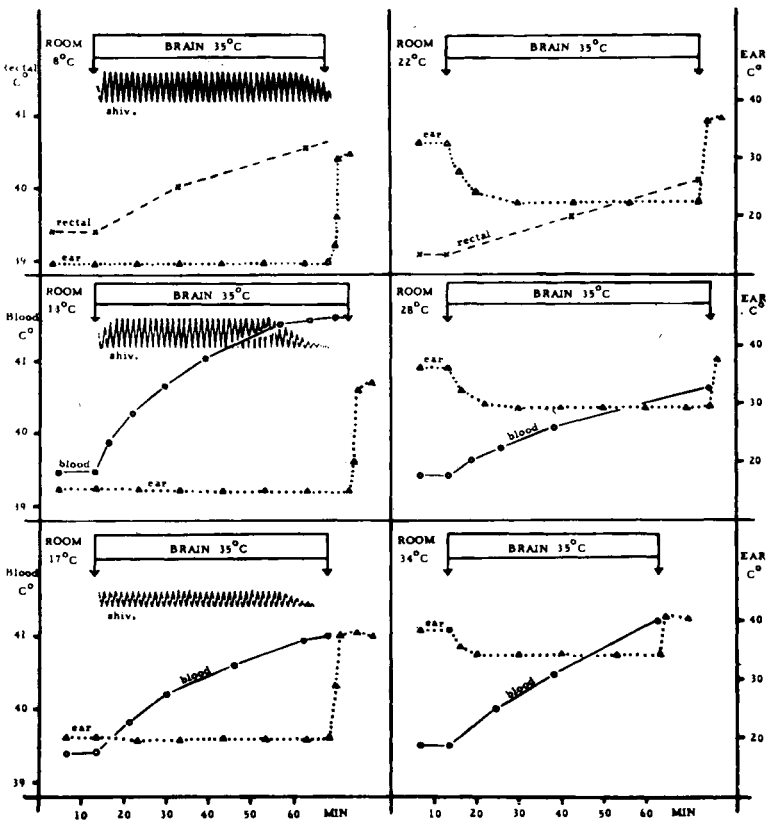

FIG. 1. Results of local cooling of the preoptic region at different environmental
temperatures in the calm goat, accustomed to the experimental conditions.
Shivering was observed only at external temperatures below 18°C.   Shivering
always stopped if the blood temperature reached 41.5 to 41.8°C.   The brain
temperature was recorded 4 mm lateral to the surface of the thermode.   (From
Andersen, Andersson, and Gale, 1962.)

known to activate the release of thyroid hormone by way of the hypo-
thalamo–pituitary axis (von Euler and Holmgren, 1956).   Recent experi-
ments indicate that the task of the " heat loss centre " is not a purely ner-
vous control of physical thermoregulatory mechanisms, but that this
" centre " also participates in the control of endocrine factors involved in
temperature regulation.   Following the onset of cooling of the " heat loss
centre ", goats develop a marked hyperthermia even in the absence of
detectable shivering.   This hyperthermia persists throughout cooling

periods of seven days, indicating an increased metabolism of non-shivering origin (Andersson, Gale and Sundsten, 1962a) (Fig. 4).   Studies of thyroid function in these animals have revealed that local cooling of the " heat loss centre " leads to a conspicuous release of protein bound iodine
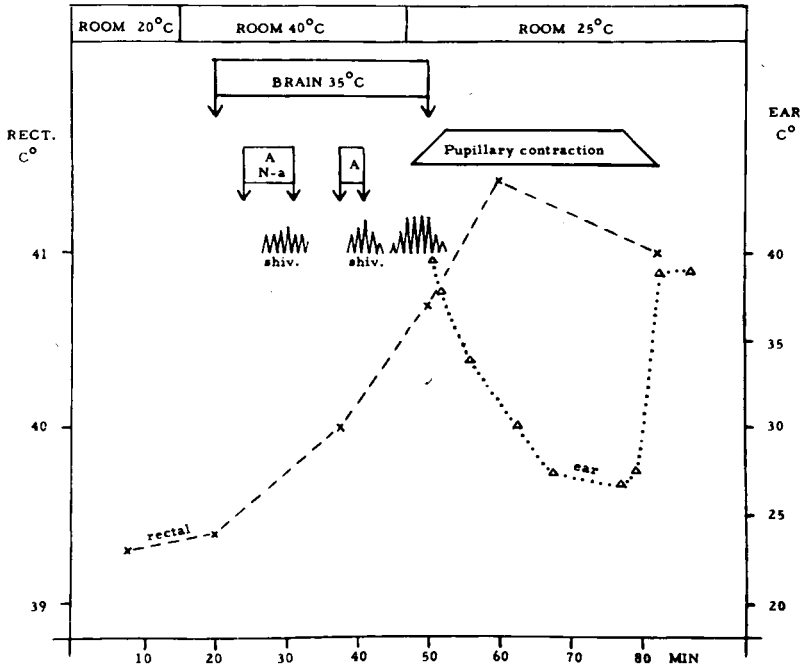

FIG. 2. Shivering induced by the intravenous infusion of adrenaline and nor-adrenaline during cooling of the preoptic area at an environmental temperature of 40°C.   In the third period of shivering, the pupillary constriction and the persistent peripheral vasoconstriction indicate that " endopyrogen " was released in this experiment.   The brain temperature was measured 4 mm lateral to the surface of the thermode.   A, adrenaline ; N-a, nor-adrenaline (Infusion rate = 1.3μg/kg b.w./min).   (From Andersen, Andersson, and Gale, 1962.)

(PBI[131]) from the thyroid.   The thyroid activation is comparable to, or even greater, than that observed when the same animals are subjected to rather severe general cold stress (Andersson, Ekman, Gale and Sundsten, 1962a and b) (Fig. 5).   If the hypothalamic control of anterior pituitary function is disturbed by lesioning the median eminence, this thyroid res-ponse to anterior hypothalamic cooling is eliminated.

Moderate local warming of the " heat loss centre " has the opposite effect to cooling (Andersson, Ekman, Gale and Sundsten, 1962c).   It blocks the thyroidal response to a general cold stress (Fig. 6) and also seems to inhibit the normal release of hormone from the thyroid (Fig. 7).   It may

therefore be assumed that the " heat loss centre " even at a normal body temperature exerts a certain inhibitory tone on the release of thyrotrophic hormone (T.S.H.) from the hypophysis. The strength of this inhibitory tone seems to increase in relation to the rise of the temperature of the " heat loss centre ", i.e. in relation to the degree of activation of central " warm detectors ".
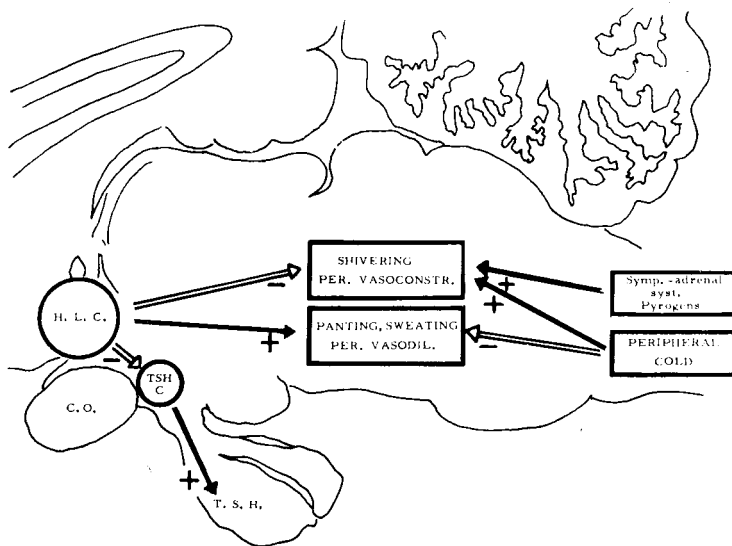

FIG. 3.   Tentative scheme depicting interaction of peripheral and central mechanisms in thermoregulation. Central " warm detectors " in the Heat Loss Centre apparently act as a brake on the shivering mechanism, as well as to mobilize various heat loss activities (vaso-dilatation, panting, sweating). Only when there is a simultaneous drive on the shivering mechanism from peripheral cold receptors or from other factors (emotional excitation, sympathetico-adrenal activation, pyrogens, etc.) does inactivation of these central " warm detectors " by cooling elicit shivering. Note also that the Heat Loss Centre seems to exert a tonic inhibition of thyroid activity via the hypothalamic-pituitary axis.

There is reason to believe that other humoral mechanisms of importance in the cold defence are also inhibited in a similar manner by the " heat loss centre " (Andersson, Gale and Sundsten, 1962b). In preliminary experiments the temperature of the rostral hypothalamus has been raised 1 to 2°C and maintained at this level when the goats are placed in a cold environment. Due to the mobilization of heat loss mechanisms and to the inhibition of shivering and other cold defence mechanisms, the core temperature of the animals falls relatively rapidly below 30°C. The hyperglycemia which otherwise is seen to develop at this low body temperature does not appear as long as the " heat loss centre " is kept active by local warming. But as soon as anterior hypothalamic warming is stopped, hyperglycemia rapidly

develops (Fig. 8).   The " heat loss centre " thus seems to inhibit the apparently humoral mechanism responsible for the hypothermic hyperglycemia. Further studies may reveal to what extent the pituitary–adrenal system and the adrenal medulla may be involved in the development of this hyperglycemia.
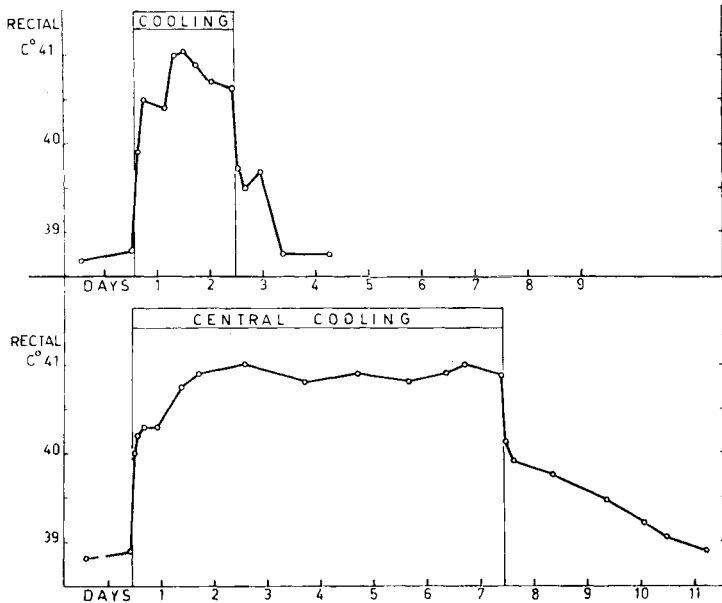

FIG. 4.   Two examples of persistent hyperthermia evoked by central cooling in the same goat.   Central cooling produced within two hours a rise in rectal temperature to 40.3 to 40.5°C ; a further rise occurred 8 to 12 hr later.   On termination of a 3-day cooling period, rectal temperature remained elevated for one day ; in contrast, following a 7-day cooling period, the core temperature did not reach normal level for 3 days.   (From Andersson, Gale, and Sundsten, 1962a.)

INFLUENCE OF HYPOTHALAMIC TEMPERATURE ON FOOD AND WATER INTAKE

During the studies of central regulation of body temperature in the goat attention has also been paid to the food and water consumption of the animals and to their alimentary behaviour.   Certain observations have been made which may perhaps be explained most easily in the light of Brobecks' (1948, 1960) " thermostatic " theory concerning the regulation of food intake.

The importance of the hypothalamus for the regulation of food and water intake has been demonstrated in several ways.   A bilateral destruction of the ventromedial hypothalamus causes obesity (Hetherington and

Ranson, 1940) which in turn is the consequence of a conspicuous hyper-phagia (Brobeck, Tepperman and Long, 1943). One explanation for this hyperphagia would be that the destruction of the ventromedial hypothala-mus intensifies the hunger " drive ". Teitelbaum and co-workers (1955, 1961) have however shown that the hunger " drive " decreases due to a
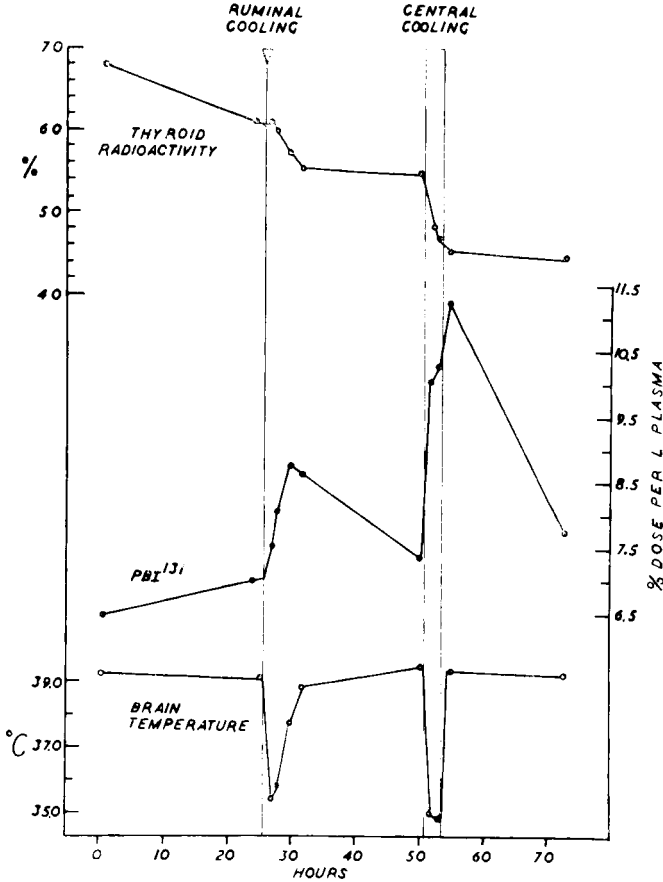

FIG. 5. A comparison of the thyroid activities caused by general cold stress (Ruminal cooling) and by local cooling of the preoptic " heat loss centre " (Central cooling). Thyroid activity is measured in per cent of the total 40 C dose of I^{131} given to the animal.

PBI^{131} = plasma protein bound I^{131}.

(From Andersson, Ekman, Gale and Sundsten, 1962b.)

lesion in the ventromedial hypothalamus, but the lesioned animals eat excessively because their ability to gain satiety during feeding has become markedly reduced. Correspondingly, the hunger " drive " disappears completely when the ventromedial hypothalamus is stimulated (Olds, 1958 ;

Hoebel and Teitelbaum, 1962). It therefore seems fully justified to call the ventromedial hypothalamus a " satiety centre " in a physiologial sense. More doubtful is whether the term " hunger " or " appetite centre " could really be used for the lateral hypothalamus. Electrical stimulation of the lateral hypothalamus may elicit stimulus bound eating (Delgado and Anand, 1953 ; Larsson, 1954) and bilateral lesions in this region may lead to temporary or even permanent aphagia (Anand and Brobeck, 1951, Teitelbaum, 1961, Morgane, 1961a). The lateral hypothalamus is however
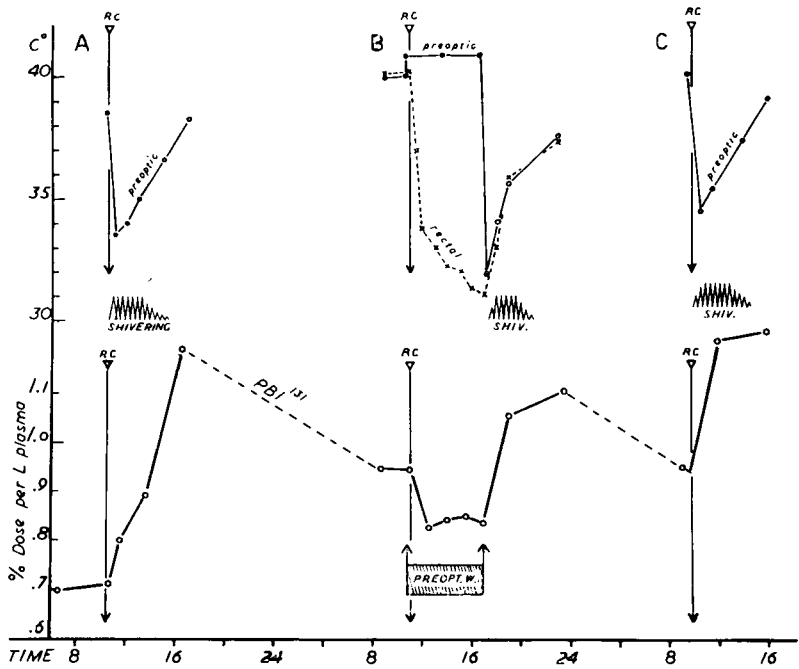

FIG. 6. A, Thyroid response (plasma PBI[131]) to ruminal cooling (RC). B, Inhibition of this response by preoptic warming (preopt. w.) (to 40.8°C). Note subsequent rise in plasma PBI[131] and onset of shivering when preoptic warming was stopped. C, Ruminal cooling repeated without preoptic warming. The dose of carrier free I[131] (60 μc) given 3 days prior to the experiment. Time of day recorded on abscissa. (From Andersson, Ekman, Gale, and Sundsten, 1962c.)

a passage for numerous nerve tracts and fibres connecting the frontal brain, the basal ganglia, and the temporal lobes with the hypothalamus and lower parts of the brain stem. The nerve cells present in this region are relatively few and scattered in this network of fibres. From the anatomical point of view the lateral hypothalamus thus by no means has the character of a " centre " and it may be questioned whether one can here talk about a " hunger centre " even in the physiological sense (Morgane, 1961b). Some of the tracts descending or crossing through the lateral hypothalamus

may thus be concerned with the coordination of alimentary motor activity. Ascending tracts, on the other hand, may be thought to transmit the urges to eat and to drink to higher organized parts of the central nervous system. This could also explain why descrete lesions in the lateral hypothalamus are often seen to cause not only aphagia but also adipsia (Teitelbaum, 1961 ; Morgane, 1961a) in spite of the fact that polydipsia as a consequence of stimulation is obtained from a more medial part of the hypothalamus, the perifornical region (Andersson and McCann, 1955b). The importance of the hypothalamus for the regulation of food intake would then be essentially to modify the hunger " drive " by determining the degree of satiety.
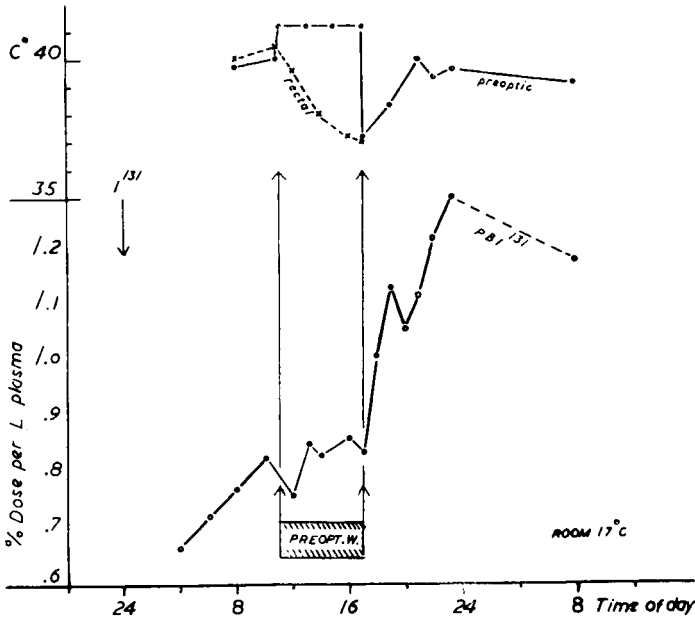

FIG. 7. Diminished release of PBI[131] into plasma during preoptic warming, followed by an increased release and a rise of body temperature on cessation of preoptic warming. Sixty microcurie of carrier free I[131] given at the arrow. (From Andersson, Ekman, Gale, and Sundsten, 1962c.)

Although stimulation and ablation experiments help to delimit regions or " centres " in the hypothalamus of importance for the control of food and water intake, such experiments generally do not reveal how changes in the internal and external environments may influence the activity and reactivity of these " centres ". Since cellular dehydration seems to be a crucial factor eliciting thirst, it is not unreasonable to suggest that the urge to drink may originate from a similar " osmoreceptor " mechanism that is regulating the release of antidiuretic hormone from the neurohypophysis

(Verney, 1947). An osmometric analysis of thirst speaks in favour of this idea (Wolf, 1950). Evidence that hypothalamic " osmoreceptors " are concerned not only with the release of antidiuretic hormone but also with the development of the urge to drink has also been produced by the observation that injections of small amounts of hypertonic saline into the medial
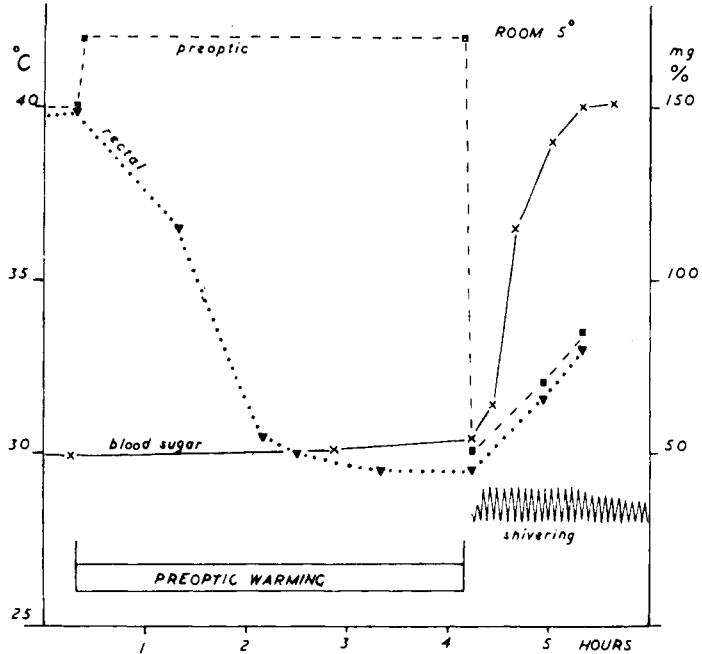

FIG. 8. Profound core hypothermia induced by local warming of the preoptic region in a goat exposed to a cold (5°C) environment. Polypneic panting and peripheral vasodilation occurred during the steep decline of body temperature to 30°C. Note that shivering and hypothermic hyper-glycemia were completely prevented during the period of preoptic warming, but occurred shortly after the cessation of warming. (From Anderson, Gale, and Sundsten, 1962b.)

hypothalamus of goats may elicit polydipsia (Andersson, 1952 ; Andersson and McCann, 1955a). But cellular dehydration is apparently not the sole factor capable of eliciting the urge to drink (Holmes and Gregersen, 1950a and b). Other changes in the internal environment such as a decreased extracellular fluid volume and a marked hyperthermia (see below) may have the same effect.

Even more complex factors seem to determine caloric intake. Two components can be separated in the regulation of food intake. There is apparently one short-term regulation working to keep the daily energy supply equal to the energy expenditure. In addition a long-term regulation works to maintain the fat depots and thus the body weight relatively constant, Various types of experimental evidence have been presented in

favour of a " glucostatic " mechanism in the ventromedial hypothalamus maintaining the short-term regulation (Mayer, 1952 and 1955). The long-term regulation may on the other hand be dependent on a " lipostatic " mechanism (Kennedy, 1953 ; Hervey, 1959).

On the basis of studies of the intimate relation that exists between body temperature and food intake Brobeck (1948, 1960) has suggested that heat may also be an important factor in the short term regulation of food intake. According to this " thermostatic " theory, hunger is reduced by a
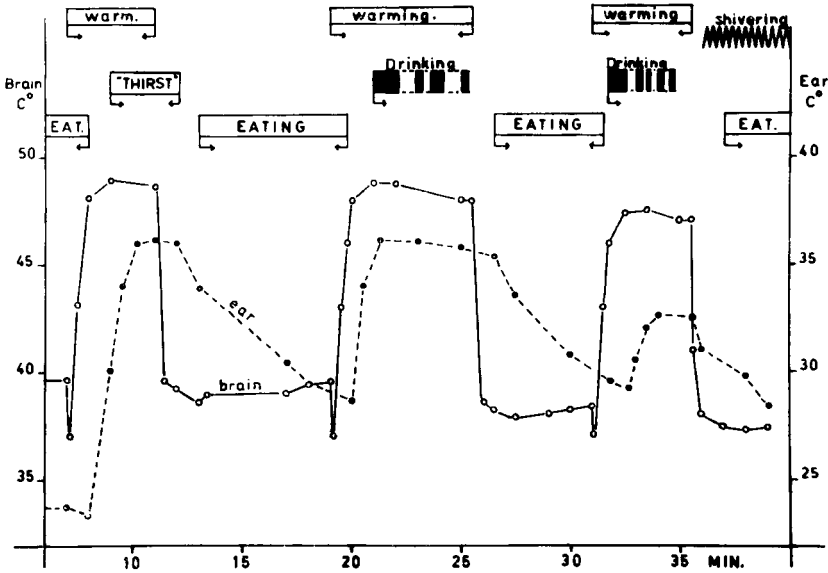

FIG. 9. Results of warming the preoptic region and rostral hypothalamus in the previously hungry animal. Brain temperature was recorded close to the surface of the thermode. The goat was fed hay at the beginning of the experiment and had free access to water except during the first period of central warming. During the periods of warming eating stopped simultaneously with the onset of peripheral vasodilatation (rise of ear surface temperature), and started again when ear surface temperature had begun to fall after discontinuation of central warming. The perfusion of the thermode with warm water induced a strong urge to drink. During the first period of central warming, when the water container was temporarily removed, it was evidenced by the animal's licking the drops of water coming out of the outlet tubing of the thermode (" thirst ") and later by the repeated drinking of large amounts of water during the periods of central warming. (From Andersson and Larsson, 1961.)

rise in temperature in the ventromedial hypothalamus (the "satiety centre") or by stimulating heat sensitive neurons in the anterior hypothalamus (the " heat loss centre "). Observations made in experiments involving warming and cooling of the preoptic region and anterior hypothalamus of the goat speak in favour of the latter possibility (Andersson and Larsson, 1961 ; Andersson, Gale and Sundsten, 1962a). Warming of the anterior

hypothalamus has thus been found to inhibit food intake and elicit thirst (Fig. 9). Cooling of the same region of the brain has to a certain extent
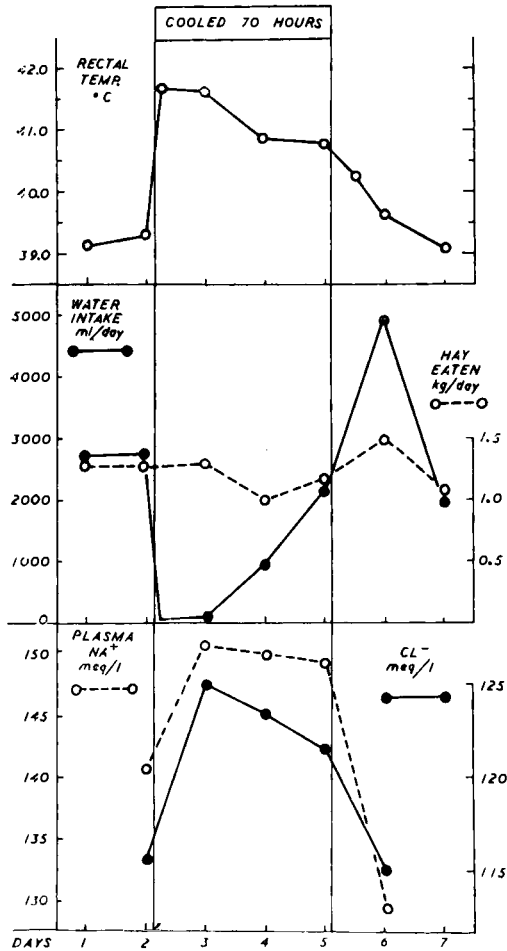

FIG. 10. Effect of chronic preoptic cooling on alimentation in a goat. During 70 hr of central cooling, water intake was totally abolished for the first 40 hr. Drinking remained reduced thereafter, the animal failing to make up its water deficit (as indicated by hyperconcentration of plasma electrolytes) until after stopping of preoptic cooling. During these periods of adipsia and hypodipsia, the animal continued to eat normal amounts of hay, despite the persistence of a marked core of hyperthermia (40.8 to 41.5°C). (From Andersson, Gale and Sundsten, 1962b.)

the reverse effect. The food intake of the animals is generally not increased but it remains at a normal level during long periods of anterior hypothalamus cooling, even though there is a rise in the body temperature to above 41°C. In the goat this is a critical temperature at which food

intake is otherwise completely inhibited (Appleman and Delouche, 1958). The water intake is initially totally inhibited and later markedly reduced during periods of preoptic and anterior hypothalamic cooling (Fig. 10).

The importance of the preoptic region and anterior hypothalamus for the thermal inhibition of food intake is further indicated by the effect of bilateral lesions in this part of the brain. A goat in which the " heat loss centre " had been destroyed by proton irradiation developed adipsia but continued to eat its normal ration of hay with apparently good appetite, even when its body temperature was raised above 41°C (Andersson and Larsson, 1961). Rats with corresponding lesions when heat stressed eat twice as much as their controls but drink less water (Hamilton, 1962). Due to the higher food intake the body temperature of the lesioned rats rises two to three degrees centigrade above the controls and may reach lethal threshold.

Local warming of any part of the central nervous system may act as a nonspecific stimulus ; local cooling, on the other hand, may inhibit all neuronal activity in the close vicinity of the thermodes. Presently the possibility can therefore not be excluded that the alimentary effects of hypothalamic warming and cooling may be more or less nonspecific and not solely due to a stimulation or inactivation of neurons specifically sensitive to heat. Since the " heat loss centre " apparently also controls endocrine factors of importance for intermediary metabolism, the possibility remains that changes in the hormonal status of the animal during preoptic warming and cooling may effect an " appestate " mechanism related to carbohydrate and fat metabolism.

Nevertheless, taken together, the alimentary effects of anterior hypothalamic lesions and those due to hypothalamic warming and cooling indicate that a " thermostatic " mechanism in Brobeck's (1960) sense really exists. It may, however, mainly serve as an emergency mechanism which prevents additional caloric supply and secures the extra supply of water necessary for urgent heat loss mechanisms, when the body temperature reaches a critically high level.

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PATTERNED ACTIVITIES FROM IDENTIFIABLE "COLD" AND "WARM" GIANT NEURONS (*APLYSIA*)

A. ARVANITAKI AND N. CHALAZONITIS

THE first demonstration of the existence of specific thermoreceptor units was made by Zotterman, recording from single fibers of the lingual nerve (Zotterman, 1936; Hensel and Zotterman, 1951; Dodt and Zotterman, 1952). More recently, evidence has been made available from intracellular recordings made simultaneously from many labelled, identifiable nerve cells in nerve centers of *Aplysia* (Arvanitaki and Chalazonitis, 1957, 1958, 1961 ; Chalazonitis, 1961, 1962).
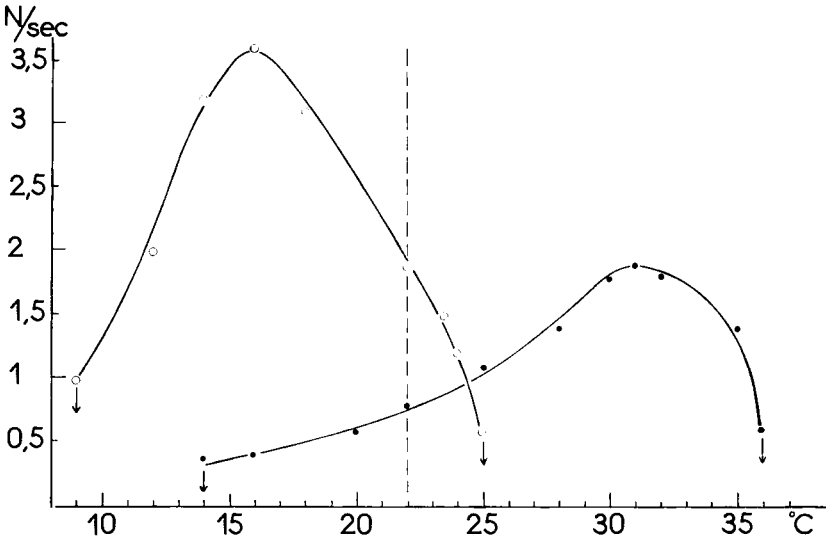

FIG. 1. Frequency (spikes per second) as function of the temperature, in a "cold" (o) and a "warm" (●) giant nerve cell of *Aplysia*. Data from simultaneous intracellular recordings. The two θ_{opt} are of extreme range values.

Under "standard" steady conditions, at 22°C, most of the cells in the isolated ganglion are autoactive in a regular way at frequencies covering a wide spectrum. By plotting frequencies versus temperature, thermal optima (θ_{opt}) were revealed. In the different cells examined, the θ_{opt} for
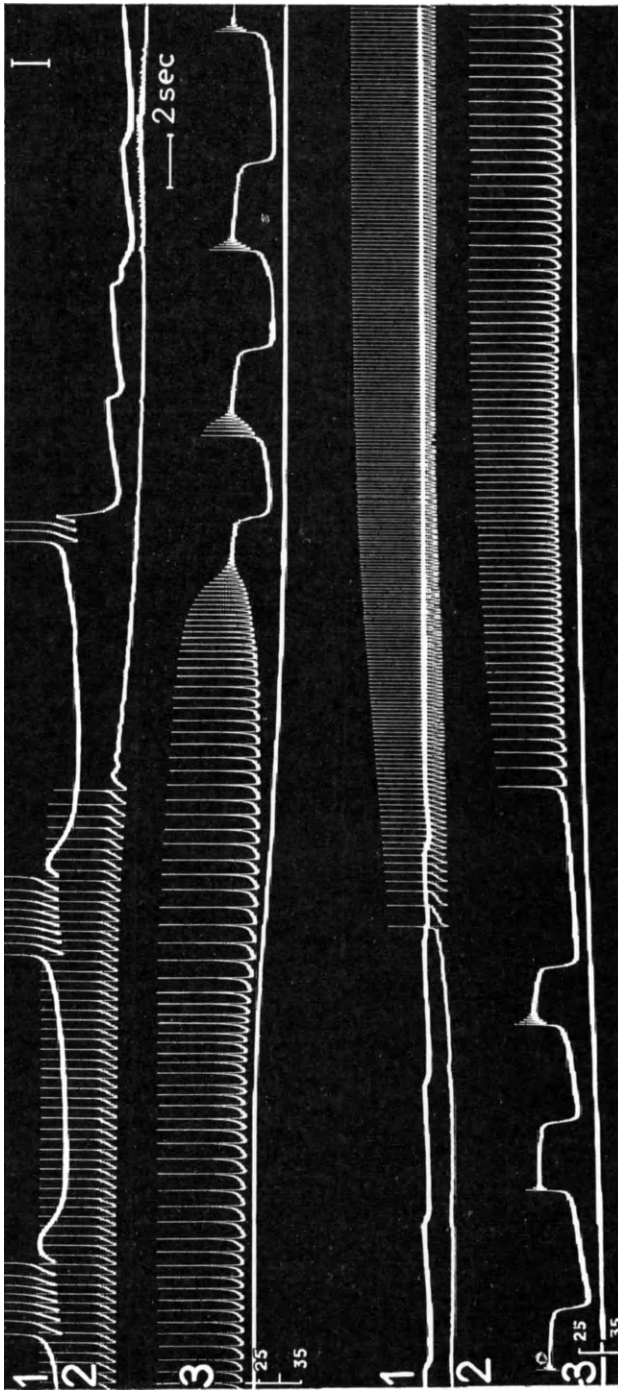
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FIG. 2. Continuous simultaneous intracellular recordings from a *Br*-type neuron (trace 1), a common " cold " neuron (trace 2) and a *Gen*-type neuron (trace 3).   Fourth trace, simultaneous recordings of temperature changes.
Upper triplet, transitions in the activity of the 3 cells on rapid warning (23° to 30°C). Lower triplet, 3 min later, transitions determined by rapid cooling to the initial temperature.
Temperature scales, on the left. Vertical bar on the right subtends 40mV.

discharge frequency were found to be distributed in between two extreme values : 16°C and 31°C. In Fig. 1 are given examples of two cells with such extreme θ_{opt}. In these, starting from 22°C, any limited increase in the temperature caused a decrease in the frequency of the low θ_{opt} nerve cell (" cold " neuron) but an increase in the high θ_{opt} nerve cell (" warm " neuron). Conversely, cooling brought about a high frequency discharge in the nerve cell of low θ_{opt}. Changes in frequency were controlled by changes in the cell membrane potential (MP) which, in "cold" neurons, was increased by warming and decreased by cooling (within certain thermal limits).

Experiments indicated that such MP changes were due to local effects of the temperature in differentiated areas of the cell membrane, spatial gradients driving intrinsic generator currents. The latter may be enhanced or abolished by appropriately directed transmembrane currents (through a second internal microelectrode). If a change in MP due to changes in other factors (e.g. partial pressure of CO_2, O_2, etc.) can, to some extent, be additive to that due to a change in temperature, one could have at least one simple interpretation of why thermal optima are dependent upon other environmental conditions.

In addition, other identifiable nerve cells display more elaborate patterned transitions in their activity, when submitted to temperature changes, than those referred to above (Fig. 2). The *Br*-type cell, for example, which at normal temperatures is autoactive in bursts occurring on rhythmic slow waves, undergoes with hyperthermia a marked hyperpolarization with superimposed repeating " inhibitory " potentials of an uncommonly large amplitude. At the same time the autoactivity of the *Gen*-type cell, continuously regular at normal temperatures, gradually turns, on warming, into a discontinuous type : the membrane potential displays square-shaped changes jumping from a hyperpolarized to a depolarized level. During the latter, high frequency spikes or oscillatory potentials are elicited. Thus the *Gen*-type cell mimics, at high temperature, the patterned behaviour specific to the *Br*-type cell at lower temperatures.

It appears to us from the above, that in addition to mere changes in spike frequency, more elaborate modal transitions in the activity of specific cells may be invoked as possible mechanisms for discrimination and supplying information at the level of primary receptor units.

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THE GUSTATORY RELAY IN THE MEDULLA*

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Prior studies of the gustatory medullary relay by Erickson (1958) and Halpern (1959) have shown that electrophysiological signs of neural activity may be recorded from the anterior solitary tract and nucleus of the rat when the anterior homolateral tongue surface is stimulated with taste solutions. The discharge of impulses resembles very closely that seen in the chorda tympani nerve under the same conditions of stimulation. The response to NaCl is typically an initial transient response followed by a decline to a steady state discharge which lasts for many minutes as long as the stimulus remains on the tongue. The time course of the discharge to sugar is characterized by a slower time rise. The relative effectiveness of different taste stimuli in exciting the medullary taste area is very similar to that seen in the chorda tympani except that in several preparations Halpern observed a relative increase in the magnitude of the response to sugar as the electrode penetrated more ventrally through the active area. The significance of this finding is still to be determined.

Erickson (1958) showed that some single units in the medulla might respond selectively to only a few of the basic taste stimuli, as, for example, positive to NaCl and HCl but not to quinine or sucrose. But many were sensitive to several of the basic test stimuli, $0 \cdot 1$ M NaCl, $0 \cdot 1$ M KCl, $0 \cdot 005$ M HCl, $0 \cdot 01$ M quinine hydrochloride, and $1 \cdot 0$ M sucrose. Not only did some units respond broadly to most of the taste stimuli, but many were also reactive to cooling and even to mechanical stimulation. Thus, individual second order taste nerve fiber units rarely displayed a narrow specificity to just one stimulus.

The present paper describes further observations on the topographic relation of the gustatory areas to somesthetic sensitivity of the mouth, head, and body regions in the rat medulla. Further studies of rat single unit activity and additional observations on the solitary nucleus of the cat will also be described.

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The procedure employed was essentially that used by Halpern and Erickson. In both the rat and cat preparations, sodium pentobarbital anesthesia was induced, the head was fixed firmly by a head holder, and the cerebellum removed to expose the floor of the IVth ventricle so that it could be explored systematically with fine insulated wire or microelectrodes in a three-dimensional manipulator.

MULTIUNIT ACTIVITY IN RAT MEDULLA

A 30μ enamelled wire yielded sufficiently localized multiunit activity to permit focal mapping of the mouth, head, and body surface as projected on the medulla. Figurine charts of the rat were constructed to show the areas from which stimulation elicited a response as observed on the CRO and audio monitor. Regions yielding responses greater than 100 μV were indicated by solid coloring, between 25–100μV by crosshatching, and less than 25μV by gray shading. Figure 1 shows the relation between the gustatory responsive areas and temperature and tactile responsivity of the mouth and other body parts at a plane $2 \cdot 9$mm anterior to the obex. The figurine plot was constructed directly from the protocol descriptions at each electrode placement; the anatomical insert was reconstructed from photomicrographs after the brains had been fixed, sectioned, and stained for histological study. The figure shows that reactivity to gustatory stimuli can appear in isolation, i.e. with no tactile or temperature responses, even with multiunit recording. This can be seen in the figure in the most dorsal point of the second penetration from the midline, second from right in the figure. Only the application of taste stimuli to the anterior tongue surface elicited neural activity at this point. As the electrode advanced, cold water on the tongue became an effective stimulus in addition to taste. Still deeper in this penetration, tactile stimulation of the lower teeth and chin became effective in addition to taste and temperature. Taste and tongue temperature sensitivity then dropped out so that only tactile responses to stimulating the lower teeth and chin remained. Finally, all tactile sensitivity dropped out. The electrode placements on either side yielded taste responses, but these occurred in conjunction with tactile sensitivity of lower teeth or chin and, in one instance in this figure, with tongue tactile sensitivity. In the fourth column from the midline, tongue tactile sensitivity was observed with *no gustatory responses*. Still more laterally, tooth, lip, and vibrissae were found. Most of the responses were evoked by homolateral stimulation but tongue tactile sensitivity in this figure was bilateral.

Figure 2 shows a similar mapping at a more caudal plane, $1 \cdot 5$mm rostral to the obex. No taste or tongue tactile sensitivity is represented here although the teeth and homolateral mucosa of the mouth are well repre-
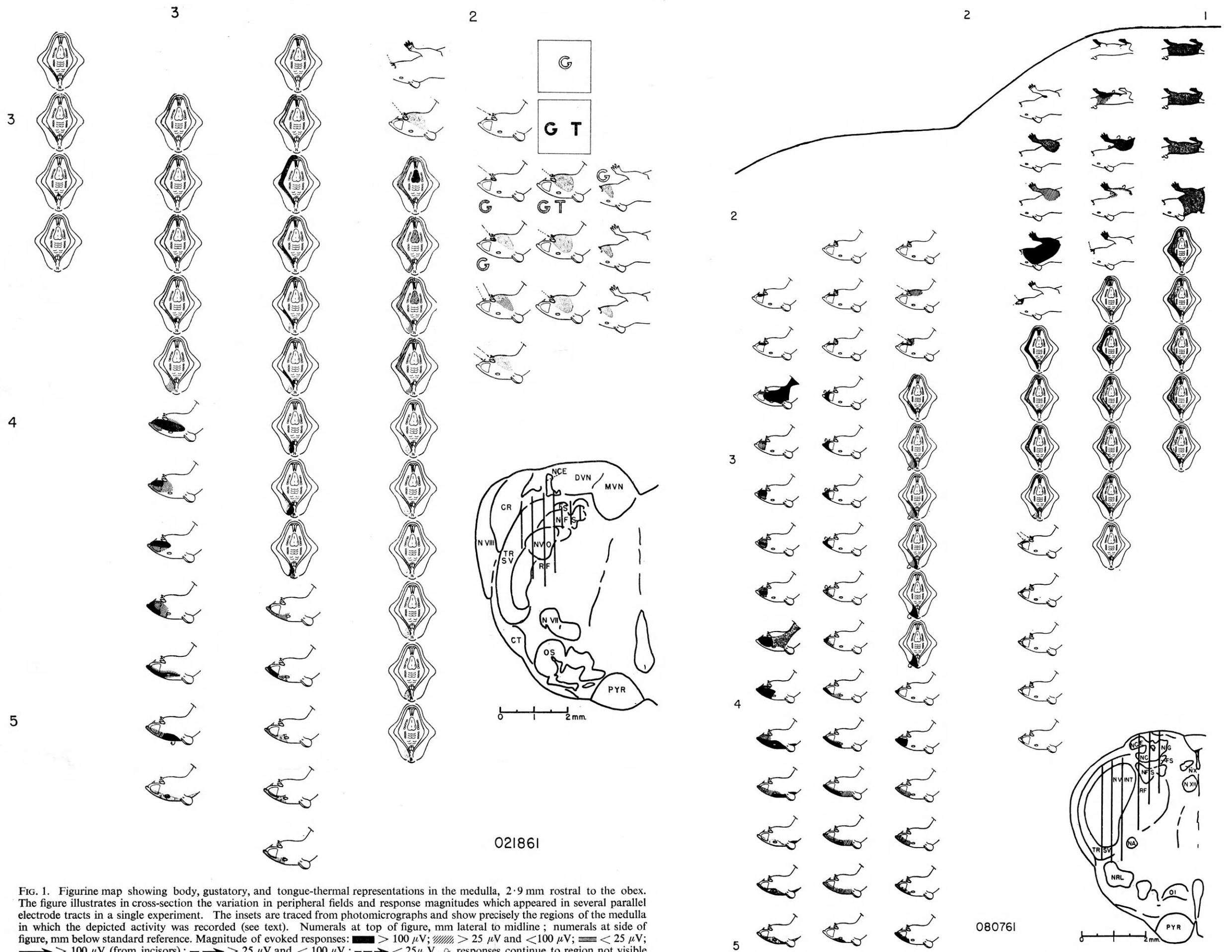
FIG. 1. Figurine map showing body, gustatory, and tongue-thermal representations in the medulla, 2·9 mm rostral to the obex. The figure illustrates in cross-section the variation in peripheral fields and response magnitudes which appeared in several parallel electrode tracts in a single experiment. The insets are traced from photomicrographs and show precisely the regions of the medulla in which the depicted activity was recorded (see text). Numerals at top of figure, mm lateral to midline; numerals at side of figure, mm below standard reference. Magnitude of evoked responses: ▓▓ > 100 μV; ▨▨ > 25 μV and < 100 μV; ▬▬ < 25 μV; ——→ > 100 μV (from incisors); – –→ > 25 μV and < 100 μV; - - → < 25μ V., ∾, responses continue to region not visible on drawing. G, gustatory responses; T, thermal responses. CR, restiform body; CT, trapezoid body; CVN, descending vestibular nucleus; FS, solitary tract; MVN, medial vestibular nucleus; NA, nucleus ambiguus; NC, cuneate nucleus; NCE, external cuneate nucleus; NRL, lateral reticular nucleus; NFS, nucleus of the solitary tract; NG, gracile nucleus; N V INT, nucleus interpolaris of the spinal trigeminal complex; N V O, nucleus oralis of the spinal trigeminal complex; N VIII, dorsal cochlear nucleus; N VII, X, XII, motor nucleus of the seventh, tenth, and twelfth cranial nerves, respectively; OI, inferior olivary complex; OS, superior olivary complex; PYR, pyramids; RF, dorso-lateral reticular zone of Torvik; TR S V, spinal tract of the trigeminal nerve.

FIG. 2. Figurine map showing body representation in a single level of the medulla, 1·5 mm rostral to the obex. Legend same as Fig. 1.

sented. In addition, the body surface, including hind and fore limbs, body trunk, as well as face and vibrissae area, are laid out in a somatotopically regular manner. The mouth sensitive areas were found not only in loci corresponding to the Vth nucleus but also in the solitary fasciculus and its nucleus. Tongue tactile responses were observed even more caudally, as far back as the level of the obex in other preparations but they were aroused only by homolateral stimulations. Taste responses from the anterior tongue stimulation were never observed this far caudally.

In comparing the figurine maps with their counterpart histological reconstructions, it should be noted that the former were drawn according to measurements on the micromanipulator during the experiment while the latter were drawn according to two different criteria. The mediolateral placement of the tracks within each of the drawings is based solely upon their relative positions as measured in the stained sections. The dorso-ventral dimensions were set up according to the relative depth below the surface at which responses were first recorded, extending for a distance equal to the depth through which they continued to be evoked. Thus, the line corresponding to the electrode path shows only the extent which yielded electrical activity. The two sets of dimensions would agree except for brain shrinkage after fixation and settling of the exposed brain during the course of experimentation.

Responses from the anterior face, eye, incisors, lips, and mucosa were obtained from the spinal nuclear complex of the trigeminal nerve. Responsive penetrations were found to extend into the region of the reticular formation medial and ventral to the spinal nucleus of the Vth nerve. Gordon et al. (1961) and Wall and Taub (1962) have made similar observations in the cat although the latter believe that the cells are not primary but second order. If so, they do not have the extremely diffuse character of other bulbar reticular neurones, for they are aroused only by sensory stimulation of the head region.

The bulbar gustatory loci in the present experiment fall within the general boundaries of the anterior tongue taste area described by Pfaffmann et al. (1961). The results indicate that the gustatory and thermal responses from the tongue arise from the nucleus of the solitary tract and, in addition, that responses from tactual stimulation of the tongue, incisors, mucosa of the mouth, lips, and jaw also may be recorded from this structure. Although purely taste loci were found, the taste reactive areas often gave tongue tactile responses like those regularly observed in the immediately surrounding placements. This is consistent with the anatomical finding that the solitary nucleus receives trigeminal fibers (Kerr, 1961 ; Torvik, 1956). Thus, the anterior solitary nucleus fits in with the somatotopic arrangement of the primarily tactile nuclei of trigeminal medullary components. It appears that the buccal cavity, including its gustatory

component, is projected in a topographical manner consistent with that of the somatosensory system.

MULTIUNIT ACTIVITY IN CAT MEDULLA

In studying the activity in the cat medullas, nichrome wire electrodes either 30 or 130μ in diameter, insulated except for the tip, were employed. The oscillograph output was monitored continuously by an audio channel and an integrator-inkwriting recorder. The magnitude of deflection of the latter provided a visual and quantifiable record of the amount of multiunit activity surrounding the tip of the electrode. Stimuli were presented either from polyethylene wash bottles or by a funnel, stopcock, and gravity flow system which flowed over the anterior part of the animal's tongue protruding from the side of the mouth. Thermal stimulation was provided by water at the approximate temperatures : warm, 38°C ; cold, 18°C ; and room temperature, 28°C. Tactile stimulation consisted of stroking the tongue with a brush. Figure 3 shows a record from an active gustatory site in the cat's medulla.

The form of the responses obtained were similar to those observed in the cat periphery (Liljestrand and Zotterman, 1954 ; Pfaffmann, 1941 ; Zotterman, 1935). The response to $1 \cdot 0$ M sucrose solution was no greater than that to distilled water. This response to distilled water, often referred to as " water taste " (Zotterman, 1958), was especially strong following HCl and KCl.

In the five penetrations in which the relationship among tactile, chemical, and thermal responses was studied quantitatively, responses to taste stimuli usually appeared most dorsally ; tactile responses were never observed dorsal to the most dorsal chemical response. Definite chemical responses occurred in two far medial electrode placements where no tactile responses were observed at any depth, but in other instances both tactile and chemical responses occurred at the same site. The relationships of the responses to chemical, thermal, and tactile stimuli in a single track are shown in Fig. 4. The maximum responses to thermal and chemical stimuli occurred at the same depth in the three preparations tested ; the maximum responses to tactile stimulation occurred at least $0 \cdot 2$ mm more ventrally.

The maximum extent of the taste area measured $0 \cdot 7$ mm rostro-caudally, $0 \cdot 4$ mm medio-laterally, $0 \cdot 8$ mm dorso-ventrally. The center of the chemically responsive area, corrected (Horsley and Clarke, 1908) for a cat skull 880 mm long and 42 mm wide, was located $5 \cdot 2$ mm rostral to the obex, $3 \cdot 2$ mm lateral to the midline, and $2 \cdot 4$ mm ventral to the surface of the medulla. According to Wall and Taub (1962), the cat's nucleus oralis of the trigeminal nucleus is dominated by tactile sensitivity

of the mouth and tongue at a level 5 mm anterior to the obex, 3·0 to 5·0 mm lateral to the midline, and at a depth of 2 + mm. Our chemically sensitive region lies at the same level but just dorsal and medial to the tongue tactile region. Thus, the gustatory area, somatotopically considered,
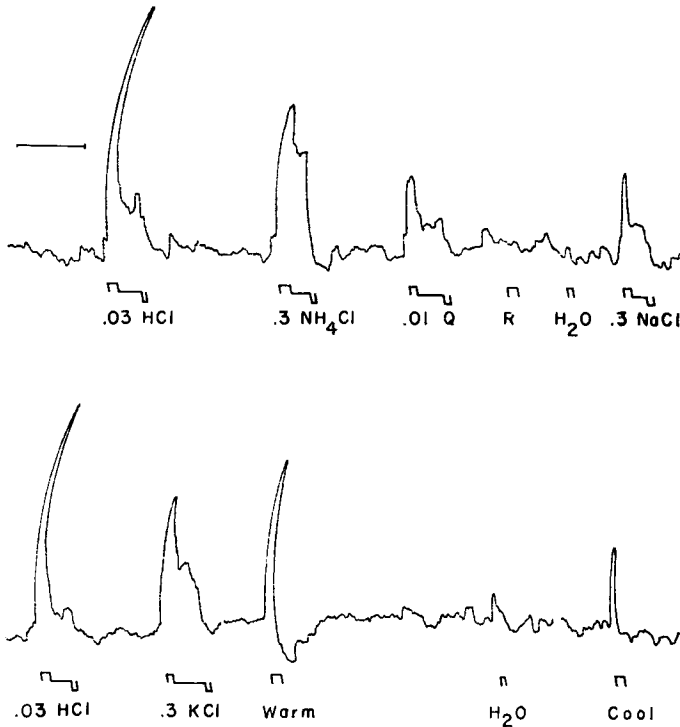

FIG. 3. Summated neural response to different stimuli. The entire record represents a continuous recording in a single preparation extending over 21½ min. The upward deflections of the markers under the record indicate duration of stimulus flow over the tongue, the downward deflections represent duration of the wash, and the lines connecting the two represent the time during which the solution rests on the tongue. The numbers refer to the molarity of the solutions. Q is quinine hydrochloride and R is Ringer's solution. The temperature of all solutions was approximately 28°C except for warm, distilled water at approximately 38°C, and cool, distilled water at approximately 18°C, as indicated. Time mark in upper left corner above the trace indicates 1 min.

borders and overlaps the tongue tactile zone but the two sensitivities are not coextensive. This agrees with the observations just described for the rat, in which both somesthetic and taste sensitivity were systematically mapped in the same animal.

Frozen sections of the medullas were made at 15, 25, or 50 μ and stained with Luxol blue and cresyl violet (Klüver and Barrera, 1953). This combined stain made it possible to differentiate the medial and lateral subnuclei

of the solitary tract (Allen, 1923 ; Brodal *et al.*, 1956 ; Torvik, 1956 ; Winkler and Potter, 1914) throughout most of the area studied. In Fig. 5, a section containing two 130 μ electrode paths is shown. Gustatory activity was observed in the left electrode path, shown here ending at the bottom of the solitary tract. In adjacent sections, this path was seen to
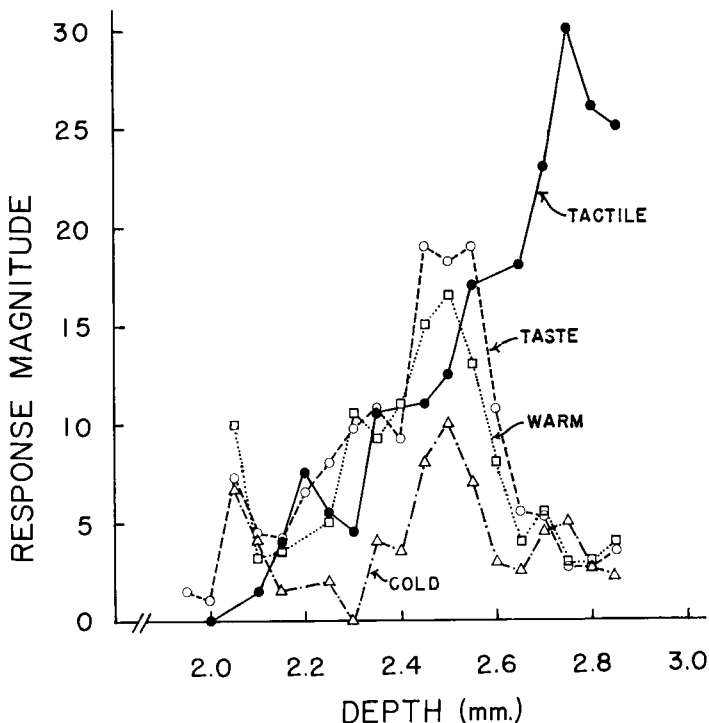

FIG. 4. Maximum relative summated neural response to chemical, thermal, and tactile stimuli as a function of electrode depth in a single electrode track. The response magnitude is measured from the prestimulus level to the highest point reached by the response ; depth of electrode tip below surface of the medulla was taken from the micromanipulator co-ordinates. The stimuli were : taste, 0·03 M HCl at approximately 28°C ; warm, distilled water at approximately 38°C ; cold, distilled water at approximately 18°C; and tactile, stroking the tongue with a camel's hair brush soaked in distilled water at 28°C.

pass through the lateral edge of the lateral solitary subnucleus. The other path, cutting through the medial edge of the medial solitary subnucleus, showed no gustatory activity. In a total of some 50 electrode penetrations studied histologically, 20 positive responses (11 definite, 9 questionable) were seen to pass through the solitary tract or its lateral subnucleus or to interrupt fascicles entering the solitary tract. Of 30 unresponsive place-ments, 8 were observed to penetrate the solitary tract or lateral subnucleus, 8 were located in the medial subnucleus, and 14 were located in other

structures of the medulla.   Thus, although all responsive penetrations
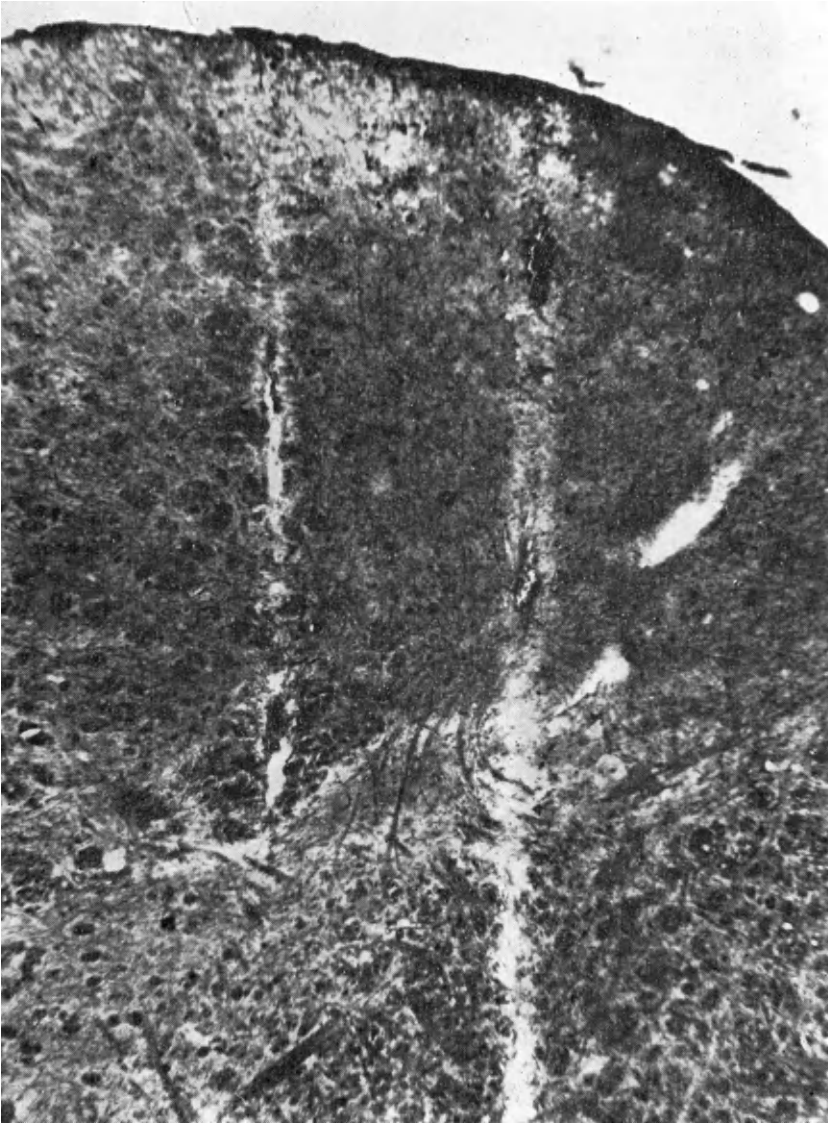passed through the solitary tract and its nucleus, not all penetrations into

FIG. 5.   Photomicrograph of frozen section of cat medulla 25 μ thick, stained
with Luxol blue and cresyl violet.   Magnification 87 ×.   See text for
explanation.

these structures yielded responses.   This might be explained by too caudal
a placement in some of the penetrations since only the rostral portion of

the solitary tract and nucleus seem implicated in anterior tongue taste sensitivity. No gustatory responses were observed either from the medial subnucleus or other medullary structures.

SINGLE UNIT ACTIVITY IN RAT MEDULLA

Further study of the activity of single second order taste units in the rat is continuing to produce interesting results. The activity of these units was recorded by a platinum-plated indium microelectrode which " floated " on a spring mounting (modified after Burns and Robson, 1960). This
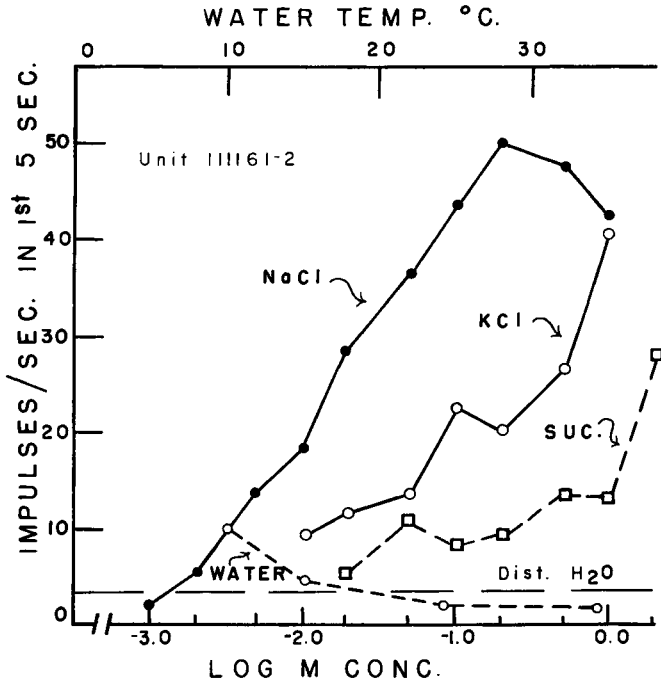

FIG. 6. The action potential frequency of a single taste unit to chemical and thermal stimulation of the tongue. The axis, " Water Temp. °C ", refers only to the curve labelled " Water ". The average level of activity to distilled water is plotted as a horizontal dashed line parallel to the x-axis.

eliminated sudden injury to the cell membrane as a result of the medulla's periodic pulsations and permitted recording from the same single neuron for periods of four or more hours. The chemical solutions, held in a container above the animal, flowed down through a tube to stimulate the rat's exposed tongue with a fine stream of fluid. Distilled water was always used to rinse the chemical solutions from the tongue. Tactile stimulation with fine monofilament hairs and thermal stimulation with

tap water (9°–37°C) were routinely employed after a unit responding to chemical stimulation had been isolated. A Grass kymograph camera photographed the action potential traces.

Figure 6 shows functional relations between the average frequency of firing and the concentration of the chemical stimulus for one unit. The response to sodium chloride is a logarithmic function of concentration, until a decline occurs with the strongest concentrations. A decline in the response to strong NaCl, similar to that found in this single unit, has also been observed in some single chorda tympani fibers (Pfaffmann, 1955). Potassium chloride and sucrose were also effective stimuli. The responses to HCl, Na saccharin, and quinine hydrochloride (not shown in this figure) were about equal in magnitude to those of the KCl function. The activity to water of different temperatures indicates that there was a slight depression to warm tap water and a significant response to cool tap water at 10°C. Mechanical stimulation of the tongue resulted in a phasic discharge.

This neuron is similar to many of the rat's second order taste units in that it responded to a number of different chemicals as well as to cooling and mechanical stimulation of the tongue. How does the nervous system detect which of these stimuli activated the single unit ? Using an average frequency measure and considering only this fiber, any one of the six chemicals applied could have produced a frequency of 20 impulses per second. If the cells are not highly specific to certain chemicals, perhaps there are clusters of cells with similar sensitivity profiles. Data bearing on this possibility are shown in Fig. 7. This is a three-dimensional array of 26 cutouts, each representing the response of a single taste unit, tested with each of the six stimuli shown on the front of the model. The vertical height of the black and white bars indicates the response magnitude to the particular stimulus. (No special significance should be attached to the order of presentation of these histograms in the model.) It is apparent that there are no particular clusters or groups of histograms that might correspond to " fiber or cell types ". Another analysis was attempted to determine whether any significant specificity of single taste units to a particular chemical could be demonstrated. The tongue was treated with a water extract of *Gymnema sylvestre*, which in human psychophysical studies produces a selective depression of the sensitivity to bitter and sweet chemicals. Yet, no selective depression of firing was found here, only a generalized depression in the response of single units to all chemicals.

The most impressive difference among the 26 units is their overall level of reactivity. If a unit was relatively insensitive to one of the taste stimuli, it was similarly unresponsive to other chemicals, and conversely, if it was highly sensitive to one chemical, it was highly sensitive to most others. At the present time, it is not clear why there should be such a wide range of

overall sensitivity. Perhaps a larger array of units is necessary to show any systematic clustering that might be correlated with the different stimuli or some alternative method of analysis is needed (see Erickson's correlation matrix method on page 205).
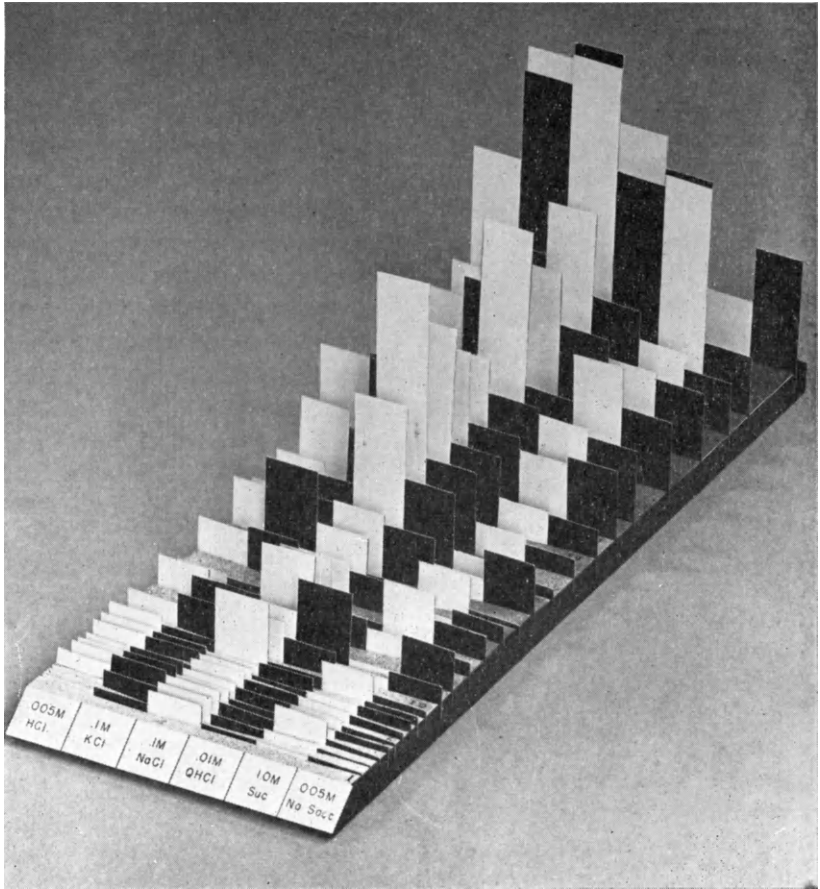

FIG. 7. Photograph of a three-dimensional model of twenty-six histogram cutouts arranged for visual presentation in terms of increasing magnitude of response. Each cutout represents a single taste unit. The test stimuli for all units are presented on the front of the model. The absolute magnitude of the response can be estimated from the response of the last unit, which was $33 \cdot 2$ impulses/sec (to $0 \cdot 005$ M Na saccharin), and from the maximum response of the first unit, which was 4 impulses/sec to $0 \cdot 1$ M NaCl.

Not only do these single taste units typically respond to a wide variety of chemicals but they often respond to cooling or mechanical stimulation of the tongue. Thirteen out of 21 taste units tested responded to mechanical

stimulation of the tongue, while 21 out of 24 units responded to cool tap water (9°–12°C).   In fact, from the present data, the generalization could be made that any given second order taste unit in the rat will respond to cooling of the tongue with water, provided the unit has a certain minimal level of reactivity to chemical stimulation.   These observations seem at variance with the results with wire electrodes (see Fig. 1) showing that certain loci are primarily responsive to gustatory stimuli.   Such areas give relatively little response to cooling or brushing the tongue, whereas other
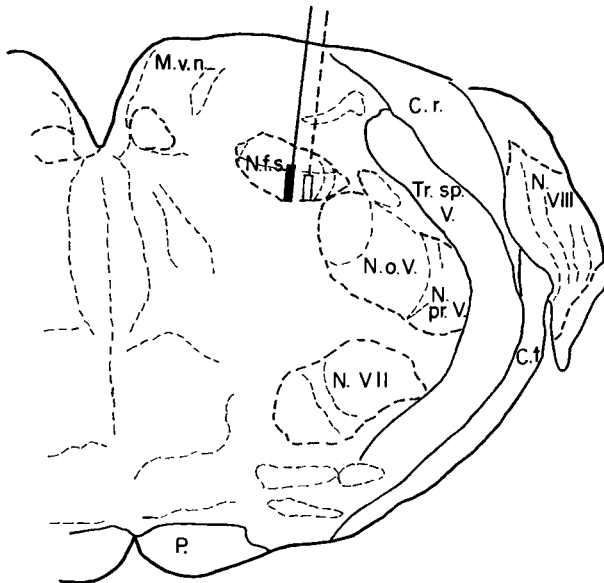

FIG. 8.   Histological loci of microelectrode tracks in animal 10,361.   Magnification 22·8 ×.   Thirty micron frozen section, cresyl violet stain.   C.r., restiform body ;   C.t., trapezoid body ;   M.v.n., medial vestibular nucleus ;   N. VII, motor nucleus of seventh cranial nerve ;   N. VIII, dorsal cochlear nucleus ; N.f.s., nucleus of the solitary tract ;   N.o.V., nucleus oralis of spinal trigeminal complex ;   N.pr.V., main sensory nucleus (nucleus principalis) ;   P., pyramids ; TR.sp.V., spinal tract of the trigeminal nerve.

contiguous points may give definite responses to both tongue tactile and temperature stimulation as well as taste.   Two factors might account for this apparent discrepancy.   In the first place, not all gustatory units respond to temperature or touch.   Thus, the density of units so activated is relatively less than that activated by taste solutions.   Secondly, the relative frequency of response to the so-called " inadequate " touch and temperature stimuli is often lower than that produced by taste solutions in the same elements. In a sense, the tactile and temperature responses may well be lost in physiological background with gross electrodes, whereas single unit recording may be more effective in picking up moderate responses.   As

the wire electrode advances from a primarily gustatory area, the density of neural response to temperature and tactile stimulation will increase due both to an increase in the number of units stimulated by touch and their greater frequency of firing so that the electrophysiological evidences would become more apparent. As the electrode advances still further, zones of overlapping tactile and taste sensitivity gradually give way to areas primarily tactual in nature until taste can no longer be recorded. In the center of the trigeminal nucleus, the response to tactual stimulation of appropriate face parts is much stronger and obvious.

For those electrode sites that could be histologically verified, the results invariably indicated that taste units lay within the solitary nucleus or just ventral to it. Figure 8 is a tracing of a transverse section through the medulla showing the position of two electrode tracks. Nearly the full extent of the medial electrode track, in which three taste units were isolated (within the black rectangular area), was visible in this one section. For one unit, the receptive area was determined, which proved to be a small point on the anterior tip of the tongue. This agrees with the histological results of Fig. 8, which show the track was in the very anterior portion of the solitary nucleus, to which the anterior tongue projects (Torvik, 1956). One of these three taste units had the lowest quinine threshold that was obtained during the course of this investigation $(0 \cdot 00006 \, \text{M})$. The open rectangle of the lateral track shows the location of single units responding solely to tactile stimulation of the tongue.

SUMMARY

1. Multiunit recording in the medullas of rats and cats in the anterior solitary tract and its nucleus yielded responses to the application of taste stimuli to the anterior tongue. Histological analysis verified the location of the taste reactive areas in the anterior solitary tract or associated nucleus.

2. The taste areas of the medulla were contiguous to, and often overlapped, areas sensitive to tactile and temperature sensitivity of the tongue and general mouth areas which bore a systematic relation to the somatotopic layout of the head and body tactile sensitivity.

3. Single unit recording in the rat medulla showed that many units displayed a generality of response to a number, if not all, taste stimuli used. In addition, many taste units could also be activated by cooling and by mechanical stimulation. However, differences in relative frequency of discharge were found among different chemical stimuli, but no simple grouping or classification of cell types was apparent.

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