EVIDENCE FOR THE CHEMICAL OLFACTORY CODE IN MAN

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A Biochemist's Approach to Olfaction

The sense of smell involves the interaction of a *bio*logical detector, the nose, with a *chem*ical stimulus, the odor. Hence the most logical methods to attack the problem would appear to be those of *biochemistry*. Although the human nose may not be the most sensitive or the most discriminating, it probably ranks first in economic importance. Yet, by the same token it is also the most inaccessible to direct experimentation by the usual neurophysiological or biochemical techniques.

Nevertheless, a classical biochemical principle is showing considerable promise in unraveling the odor problem. Most of the chemical steps in pathways of intermediary metabolism were worked out by taking advantage of mutations. A strain of microorganisms so afflicted has lost the genetic capacity to produce a certain enzyme, so the normal substrate of this enzyme accumulates, reaching a level at which it can be chemically identified. Admittedly, most such mutations are artificially induced by x ray or UV radiation, but some occur naturally, and man himself is not immune to this phenomenon. Occasionally such inborn errors of metabolism[1] are actually diagnosed by the physician's sense of smell, as in the recently described isovaleric acidemia, in which the enzyme isovaleryl coenzyme A dehydrogenase is defective, and the unfortunate young patient reeks of stale cheese.[2]

Paradoxically, about one physician in fifty would probably not at first believe that anything was wrong with the child, because the doctor himself happens to have a mutated nose that cannot perceive isovaleric acid or related short-chain fatty acids. Here is our chance to disentangle the olfactory problem. This biochemical defect of odor blindness or *specific anosmia* is relatively common and occurs in many varieties. As a working hypothesis, I assume that the olfactory epithelium of an affected person lacks the specific receptor protein for detecting one whole family of odorants that belong to one of the *primary odors*.

Although not couched in biochemical terms, this concept was first clearly enunciated by the physicist Marcel Guillot a quarter of a century ago[3] in his paper entitled "Anosmies Partielles et Odeurs Fondamentales." For the past eight years I have been elaborating the techniques to exploit this phenomenon, and I have been enabled to map out quite thoroughly the domains of two such "odeurs fondamentales."

The Mapping of Two Primary Odors

The first primary odor to be worked out in detail was the "sweaty" odor developed by isovaleric acid and its congeners. The starting point was the casual observation by a biochemist that he, personally, could not smell the generally disagreeable isobutyric acid. I found that about two percent of people have the same deficiency (or advantage!). The same people also have varying degrees of difficulty in detecting other short-chain fatty acids. The deficiency is estimated by measuring

the odor-detection threshold of each subject for each odorant. The average threshold concentration for odor-blind subjects is substantially higher than the average threshold for normal observers. The difference between the two group thresholds measures the *primacy* of the odor. The higher the threshold difference between normal and anosmic observers, the more closely does the test compound approach the true primary odor. The method and results have been described in detail elsewhere.[4, 5]

The data for this type of anosmia are conveniently summarized in FIGURE 1. Considering first the normal or straight-chain acids, the anosmic deficiency rises to a plateau between 4 and 7 carbon atoms. Hence molecular size is an important determinant of odor quality. At a given molecular size, for example, with the four

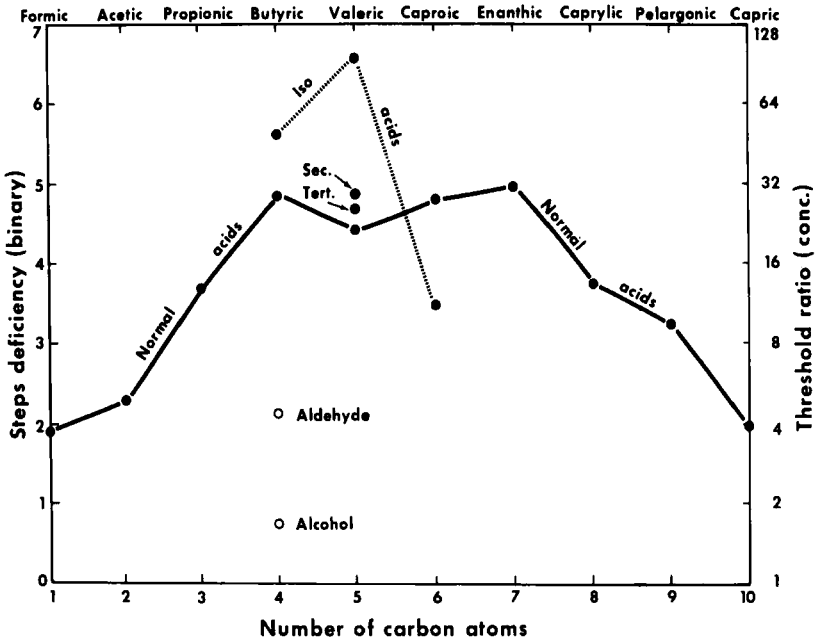
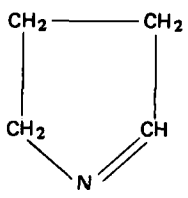


FIGURE 1. Specific anosmia to isovaleric acid and congeners.

valeric acid isomers, the degree of anosmia was greatest with isovaleric acid. This shows that molecular shape can influence the intensity of odor. Among compounds so far tested, isovaleric acid has shown the largest anosmic deficiency in this odor class, and so it is considered to epitomize the primary odor of sweatiness. Finally, even though the size and the shape of a molecule are appropriate, as with isobutyraldehyde or isobutyl alcohol, the sweaty primary odor is absent when the functional group is wrong. Evidently the functional carboxylic acid group, the molecular size, and the shape of the molecule all contribute in determining whether a molecule can elicit the sweatlike odor.

Work on the second primary odor is nearly completed. The initial observation was that about 20% of people, although having an otherwise normal olfactory ability, fail to perceive the odor of 1-pyrroline. This is a heterocyclic Schiff's base,



whose odor closely resembles that of semen. A large selection of nitrogenous bases has been tested with a panel of 29 subjects having the 1-pyrroline anosmia, and the results will be published in detail elsewhere.[6] The "spermous" primary odor is most pronounced in 1-pyrroline itself, which showed an anosmic deficiency of 6.19 binary steps. The next higher homologue, 1-piperideine, registered about five steps. Many other aliphatic and cyclic amines and imines were tested, but after purification they have only a stale fishy odor, which these anosmics have no difficulty in detecting. Evidently the molecular requirements for producing the spermous primary odor are very restricted.

I foresee no reason to doubt that the same experimental methods can be extended to the odor of musk, and to the 20–30 other known varieties of specific anosmia that probably correspond, each with a distinct primary odor.[7] Only time and money will tell. The entire collection of primary odors, together with their chemical definitions, will constitute the chemical "olfactory code." Of this we have obtained so far just a glimpse. Superimposed on this chemical coding at the receptor level there must be an elaborate neural coding in the olfactory bulb and brain, but it is unlikely that these events can be deciphered before the receptor process is understood.

Supporting Evidence for Primary Odors

It would be desirable to apply some independent experimental methods to see if they give results in agreement with the primary odors delineated by the specific anosmia method. Because of the difficulties that prevent direct experiments on the human nose, we again have to rely on psychophysical methods. I have carried out some studies on the sweaty primary odor, using odor-similarity scaling, odor confusion, and olfactory fatigue.[8]

The results for the first ten normal acids are collected in FIGURE 2. The curve derived from measurements of anosmic deficiency is repeated for comparison. Isovaleric acid (suitably diluted) was employed as the reference standard. When the other acids were presented as unknowns to a panel of normal observers, the odor-similarity ratings on a scale of 0–8 showed fair agreement with the anosmia curve. Another approach was to dilute each acid until the odor intensity equals that of the isovaleric acid standard, and then to see if the subjects could tell the difference. Substantial confusion occurred only with the four and five carbon acids. The results obtained in a fatigue test were still more selective. After a single-sniff adaptation of the nose with isovaleric acid at 64 times threshold, only the valeric acid isomers (and isobutyric and isocaproic acid) suffered any significant temporary increase in threshold.

It should be noted that the similarity, confusion, and fatigue tests were conducted with subjects whose sense of smell for the fatty acids is perfectly normal. Hence they tend to confirm qualitatively the identification of the sweaty primary odor achieved with the specifically anosmic observers. Nevertheless, the agreement is far from quantitative, and some explanation for this should be sought. Part of the difference may be ascribed to nonuniformity of the numerical scales used for each method. In addition, we should consider that the anosmia method probably reflects events in the initial chemical step of olfaction at the receptor surface, whereas the similarity, confusion, and fatigue results depend on differing sets of properties of the nervous pathways and higher correlative centers in the olfactory bulb and brain.

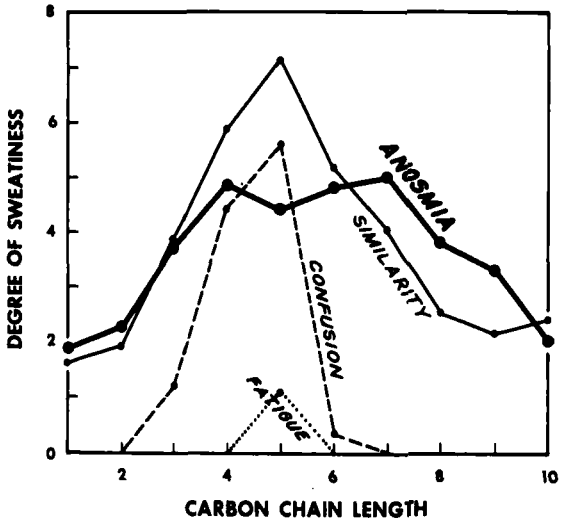


FIGURE 2. The sweaty primary odor specificity examined by four methods.

If indeed specific anosmia is due to a defect in a selective receptor protein, then, like most other inborn errors of metabolism, the defect should exhibit a rather simple recessive inheritance pattern. This has actually been found to be the case for the specific anosmia to the musk pentadecalactone, which affects about seven percent of the population.[9] Among 109 Caucasian families examined in collaboration with Dr. D. Whissell-Buechy of the University of California at Berkeley, 36 contained specific anosmics to musk (FIGURE 3). The distribution of anosmics within these families agrees very closely with what would be expected from a nonsex-linked Mendelian recessive character.

The scarcity of persons with the isovaleric acid anosmia (2%) makes an inheritance study a protracted undertaking. We have found, however, one family in which the father and his two children from the present marriage were anosmic, but his wife and her three children by a previous marriage were normal in this respect. There being plenty of data to show that the inheritance is not dominant, this family tree again supports the recessive inheritance hypothesis, the wife being a heterozygous carrier of the deficiency.

Valuable as these supplementary methods are for confirming the existence and range of a primary odor, it still remains true to say that only the specific anosmia method seems able to identify a human primary odor in the first place. This is an unsatisfactory situation, and it is to be hoped that some independent method can be devised for establishing primary odors in man, or at least that the specific anosmia method should be consolidated by animal experimentation.

CORRELATIONS BETWEEN ODOR AND MOLECULAR SHAPE

I consider that the investigations described above provide substantial support for the concept that the human sense of smell is based on a system of discrete primary odor sensitivities, each of which is independently inherited. Of course, the

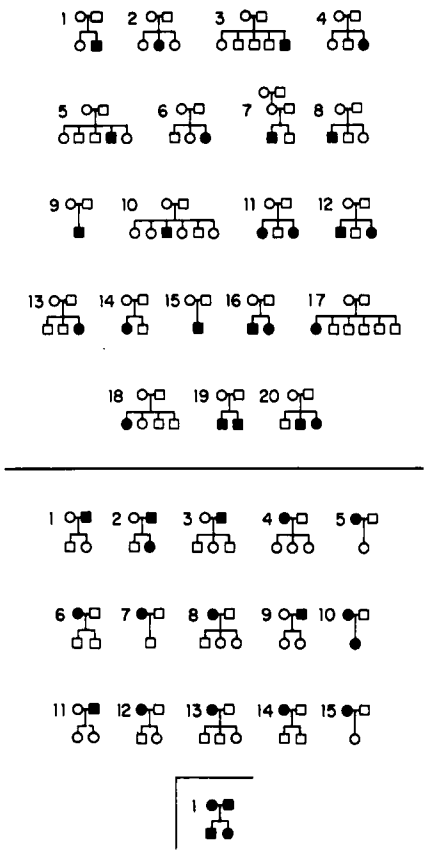


FIGURE 3. Family trees showing incidence of musk anosmia (solid symbols).

conclusion is very provisional, because only two of the expected thirty primary odors have been chemically mapped, and a third submitted to complete genetic analysis. Meanwhile, the problem that is now delineated even more clearly is the old one of finding common molecular characteristics among molecules developing the same primary odor.

Regarding the sweaty odor, it seems that the possession of the appropriate functional group (carboxylic acid) is an absolute requirement. If the functional group is correct, then the size and shape of the whole molecule determine how closely the primary odor is approached. Molecular morphology is open to quantitative measurement, and this has been achieved in the case of the fatty acids by building scale molecular models and photographing them from three directions at right angles. Each photograph can then be compared with the corresponding photo for isovaleric acid, either by graphic methods or by a computer equipped with a random-line television scanner developed by Dr. G. Palmieri at the University of Genoa. For the fatty acids a reasonably good correlation (FIGURE 4) has been observed between odor primacy and molecular size and shape measured by the PAPA scanning computer.[10] The correlation coefficient is $r = 0.80$.

A disadvantage of the above procedure is that it relies upon intuitive selection

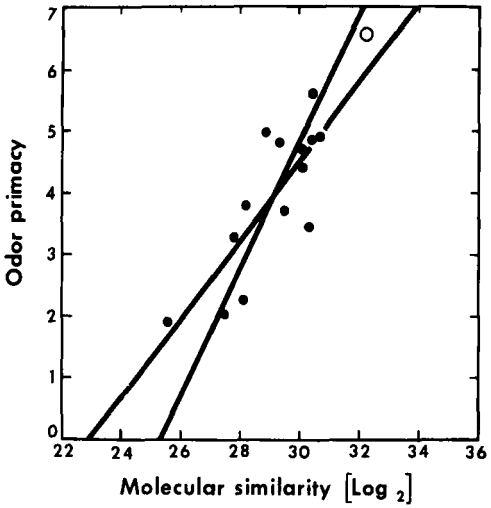


FIGURE 4. Correlation between sweaty odor and molecular shape.

of the most likely conformation when arranging the atomic units in a model of a flexible molecule. This problem can, however, be avoided by going over to a fully computerized analysis of the molecular resemblances. Dr. N. L. Allinger at the University of Georgia has examined most of the same fatty acids, adapting a computer program he has developed for finding the most likely molecular conformation.[11] The results are in close agreement with those obtained by the PAPA machine.

The fact that odor quality does correlate to a substantial degree with molecular size and shape indicates that the olfactory receptor system is sensitive to these parameters. Hence a specific sterochemically definable proteinaceous receptor site for each primary odor remains perhaps the most attractive working hypothesis. Any competing theories of odor should be assessed by the common yardstick of the correlation coefficient, if they are to be credited with appreciable success in explaining known olfactory facts.[12]

POSSIBLE APPLICATIONS FOR THE PRIMARY ODORS

Knowledge of the primary odors and their chemical characteristics will be of rather limited practical use until all of the primaries are known. At present only two are well established, whereas the total number could be thirty or even more.[7] During the 1963 Conference[13] I presented a different line of stereochemical reasoning that suggested only seven primary odors, none of which correspond exactly with the sweaty or spermous primaries, although the old musky primary odor seems likely to be confirmed by the anosmia method. It is too early to attempt an analysis of the reasons for the difference, but the discrepancy at least underscores my insistence that at present only the specific anosmia procedure is capable of accurately identifying human primary odors.

No early answer seems possible to the question of the total number of primary odors. I can see no alternative to mapping out each one patiently by the laborious specific anosmia method, which demands at least one scientist-year of research per primary. Nevertheless, I should like to suggest a few situations where theoretical understanding of the primaries could have practical value.

Synthetic flavors are presently compounded from a list of more than 700 chemicals that are deemed GRAS (Generally Recognized As Safe). Yet very few have been rigorously tested for safety because of the appalling cost of toxicological testing (up to $1 million/compound). I have suggested that an understanding of the primary odors would permit many redundant compounds to be deleted from the GRAS list, so that toxicological resources could be focused upon those few compounds that are close to being primary odorants and that have low thresholds for perception.[14]

We suspect that the frequency of occurrence of specific anosmias varies between the Caucasian and Negro races, and possibly even between nationalities within races.[9] This finding would appear to offer a readily accessible fund of biochemical data for research in population genetics.

It is noticeable that the first three primary odors studied arouse more than a suspicion of being associated with primate and even human pheromones.[15] The sweaty and spermous odors are self-explanatory, whereas the musk odor is developed by some steroid constituents of urine.

Assessment of water- and air-pollution nuisance would be rendered more exact if it could be stated which primary odors were being offended. Some of our data on normal and anosmic observers could be useful in establishing norms of human sensitivity.

Conversely, if it is known that an offensive smell belongs to a given primary odor, then knowledge of the corresponding chemical characteristics might suggest an effective method for preventing or counteracting the problem. Specific adsorbants might even be designed to mimic the olfactory receptor site, and thereby selectively purify the effluent.

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