The Stereochemical Theory of Odor

There is evidence that the sense of smell is based on the geometry of molecules. Seven primary odors are distinguished, each of them by an appropriately shaped receptor at the olfactory nerve endings

by John E. Amoore, James W. Johnston, Jr., and Martin Rubin

A rose is a rose and a skunk is a skunk, and the nose easily tells the difference. But it is not so easy to describe or explain this difference. We know surprisingly little about the sense of smell, in spite of its important influence on our daily lives and the voluminous literature of research on the subject. One is hard put to describe an odor except by comparing it to a more familiar one. We have no yardstick for measuring the strength of odors, as we measure sound in decibels and light in lumens. And we have had no satisfactory general theory to explain how the nose and brain detect, identify and recognize an odor. More than 30 different theories have been suggested by investigators in various disciplines, but none of them has passed the test of experiments designed to determine their validity.

The sense of smell obviously is a chemical sense, and its sensitivity is proverbial; to a chemist the ability of the nose to sort out and characterize substances is almost beyond belief. It deals with complex compounds that might take a chemist months to analyze in the laboratory; the nose identifies them instantly, even in an amount so small (as little as a ten-millionth of a gram) that the most sensitive modern laboratory instruments often cannot detect the substance, let alone analyze and label it.

Two thousand years ago the poet Lucretius suggested a simple explanation of the sense of smell. He speculated that the "palate" contained minute pores of various sizes and shapes. Every odorous substance, he said, gave off tiny "molecules" of a particular shape, and the odor was perceived when these molecules entered pores in the palate. Presumably the identification of each odor depended on which pores the molecules fitted.

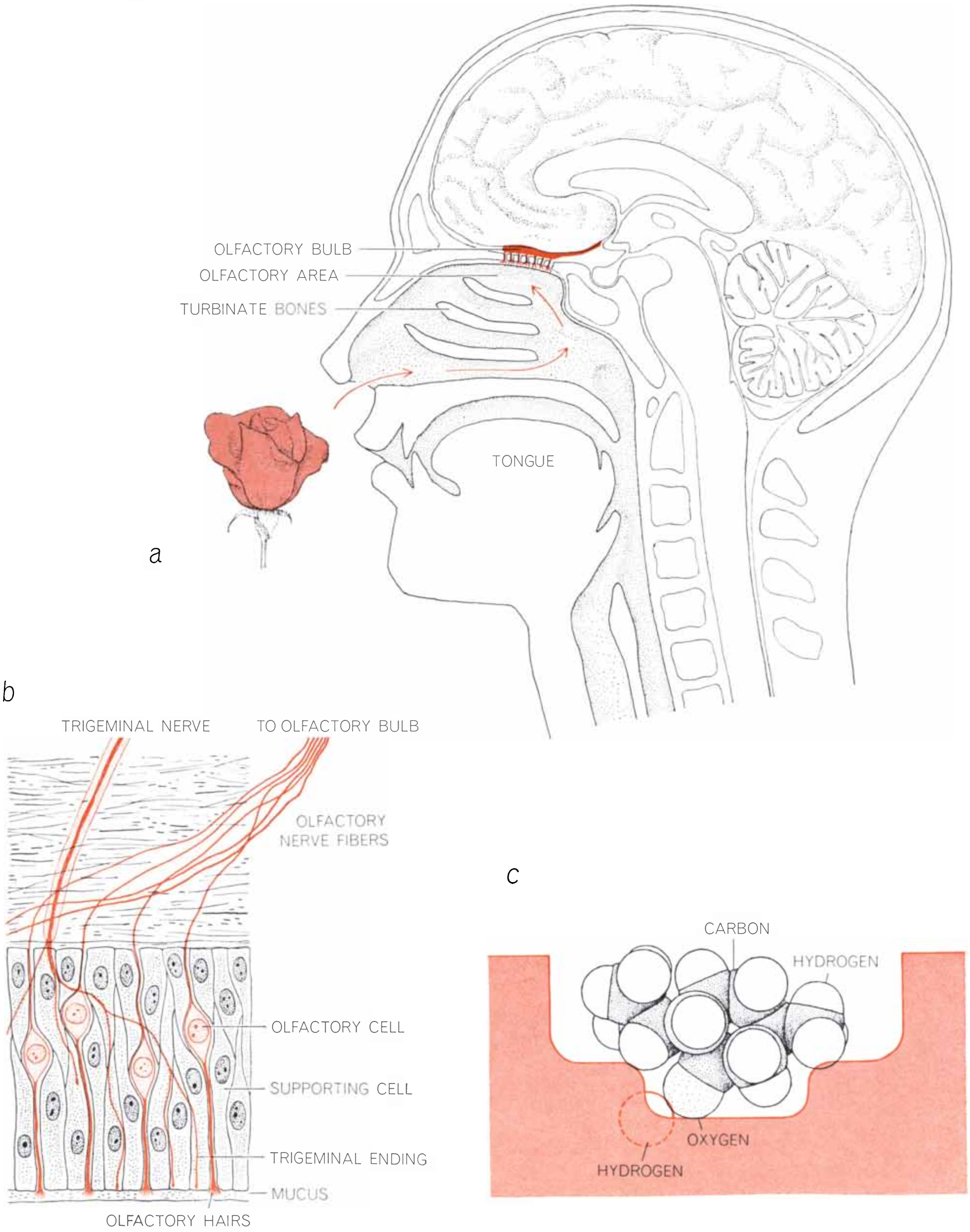
It now appears that Lucretius' guess was essentially correct. Within the past few years new evidence has shown rather convincingly that the geometry of molecules is indeed the main determinant of odor, and a theory of the olfactory process has been developed in modern terms. This article will discuss the stereochemical theory and the experiments that have tested it.

PRIMARY ODOR	CHEMICAL EXAMPLE	FAMILIAR SUBSTANCE
CAMPHORACEOUS	CAMPHOR	MOTH REPELLENT
MUSKY	PENTADECANOLACTONE	ANGELICA ROOT OIL
FLORAL	PHENYLETHYL METHYL ETHYL CARBINOL	ROSES
PEPPERMINTY	MENTHONE	MINT CANDY
ETHEREAL	ETHYLENE DICHLORIDE	DRY-CLEANING FLUID
PUNGENT	FORMIC ACID	VINEGAR
PUTRID	BUTYL MERCAPTAN	BAD EGG

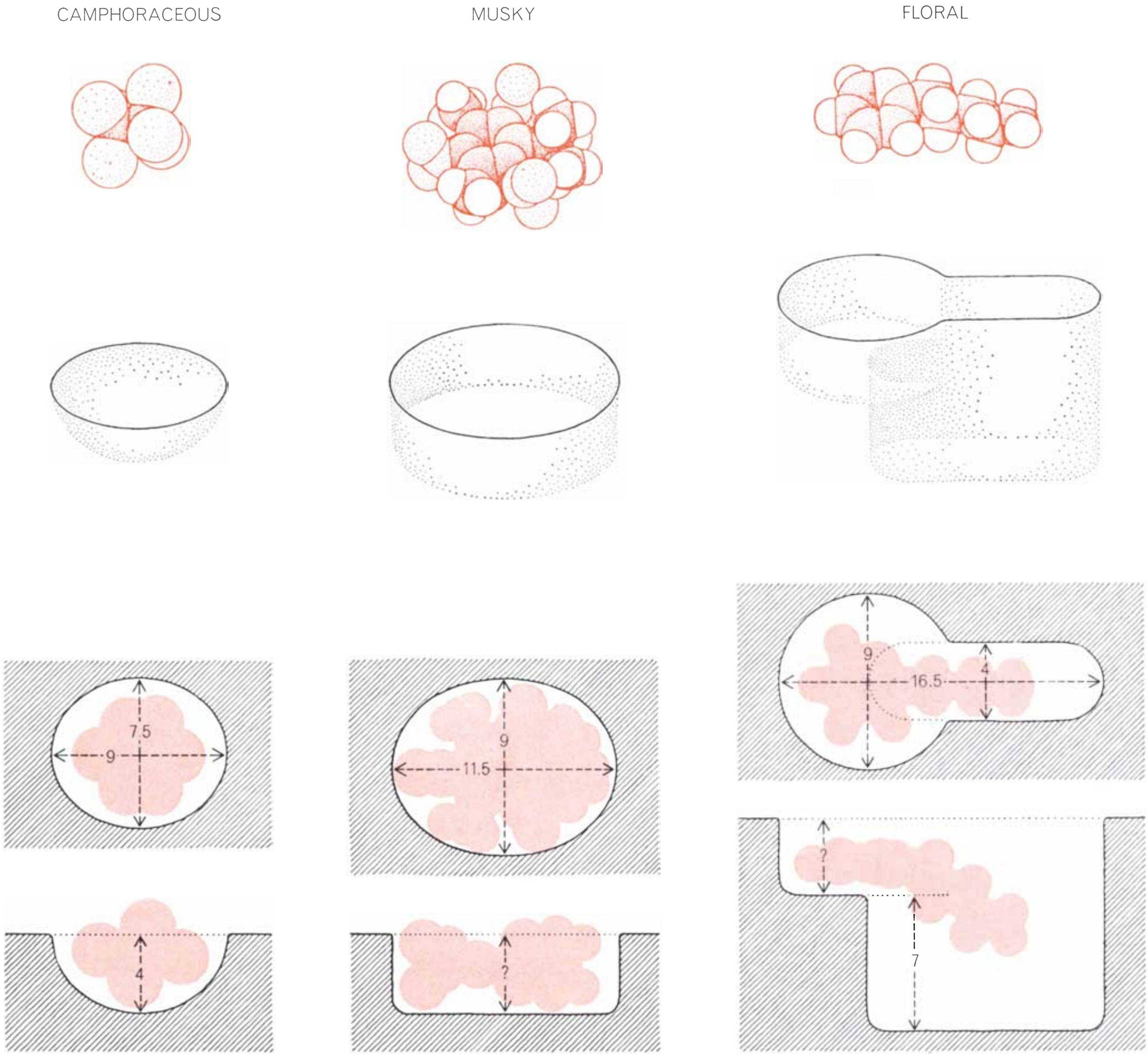
PRIMARY ODORS identified by the authors are listed, together with chemical and more familiar examples. Each of the primary odors is detected by a different receptor in the nose. Most odors are composed of several of these primaries combined in various proportions.

The nose is always on the alert for odors. The stream of air drawn in through the nostrils is warmed and filtered as it passes the three baffle-shaped turbinate bones in the upper part of the nose; when an odor is detected, more of the air is vigorously sniffed upward to two clefts that contain the smelling organs [*see illustration on opposite page*]. These organs consist of two patches of yellowish tissue, each about one square inch in area. Embedded in the tissue are two types of nerve fiber whose endings receive and detect the odorous molecules. The chief type is represented by the fibers of the olfactory nerve; at the end of each of these fibers is an olfactory cell bearing a cluster of hairlike filaments that act as receptors. The other type of fiber is a long, slender ending of the trigeminal nerve, which is sensitive to certain kinds of molecules. On being stimulated by odorous molecules, the olfactory nerve endings send signals to the olfactory bulb and thence to the higher brain centers where the signals are integrated and interpreted in terms of the character and intensity of the odor.

From the nature of this system it is obvious at once that to be smelled at all a material must have certain basic properties. In the first place, it must be volatile. A substance such as onion soup, for example, is highly odorous because it continuously gives off vapor that can reach the nose (unless the soup is im-



ANATOMY of the sense of smell is traced in these drawings. Air carrying odorous molecules is sniffed up past the three baffle-shaped turbinate bones to the olfactory area (*a*), patches of epithelium in which are embedded the endings of large numbers of olfactory nerves (*color*). A microscopic section of the olfactory epithelium (*b*) shows the olfactory nerve cells and their hairlike endings, trigeminal endings and supporting cells. According to the stereochemical theory different olfactory nerve cells are stimulated by different molecules on the basis of the size and shape or the charge of the molecule; these properties determine which of various pits and slots on the olfactory endings it will fit. A molecule of *l*-menthone is shown fitted into the "pepperminty" cavity (*c*).



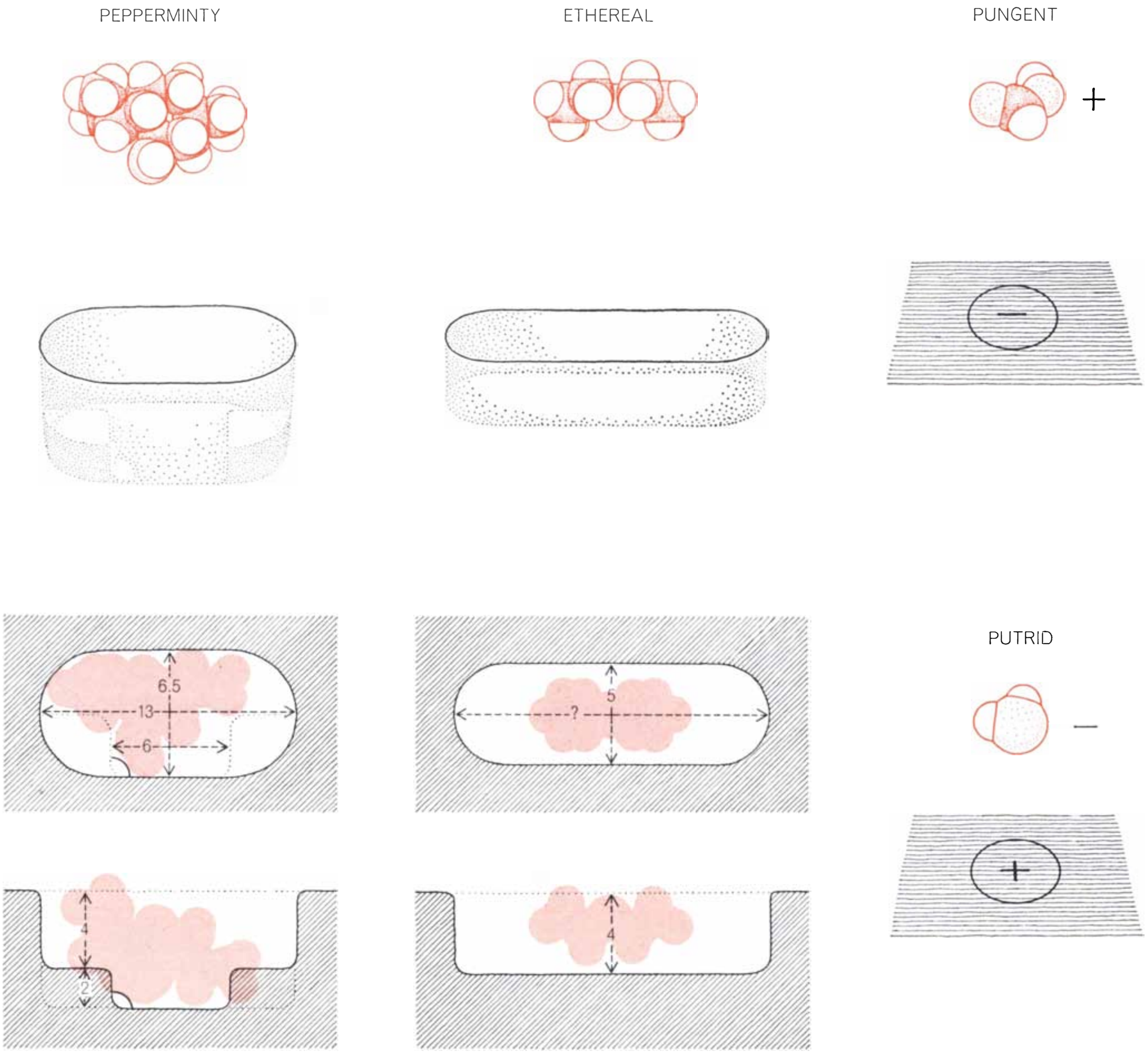
OLFACTORY RECEPTOR SITES are shown for each of the primary odors, together with molecules representative of each odor. The shapes of the first five sites are shown in perspective and (with the molecules silhouetted in them) from above and the side;

prisoned in a sealed can). On the other hand, at ordinary temperatures a substance such as iron is completely odorless because it does not evaporate molecules into the air.

The second requirement for an odorous substance is that it should be soluble in water, even if only to an almost infinitesimal extent. If it is completely insoluble, it will be barred from reaching the nerve endings by the watery film that covers their surfaces. Another common property of odorous materials is solubility in lipids (fatty substances); this enables them to penetrate the nerve endings through the lipid layer that forms part of the surface membrane of every cell.

Beyond these elementary properties the characteristics of odorous materials have been vague and confusing. Over the years chemists empirically synthesized a wealth of odorous compounds, both for perfumers and for their own studies of odor, but instead of clarifying the properties responsible for odor these compounds seemed merely to add to the confusion. A few general principles were discovered. For instance, it was found that adding a branch to a straight chain of carbon atoms in a perfume molecule markedly increased the potency of the perfume. Strong odor also seemed to be associated with chains of four to eight carbon atoms in the molecules of certain alcohols and aldehydes. The more chemists analyzed the chemical structure of odorous substances, however, the more puzzles emerged. From the standpoint of chemical composition and structure the substances showed some remarkable inconsistencies.

Curiously enough, the inconsistencies themselves began to show a pattern. As an example, two optical isomers—molecules identical in every respect except that one is the mirror image of the other—may have different odors. As another



known dimensions are given in angstrom units. The molecules are (*left to right*) hexachloroethane, xylene musk, alpha-amylpyridine, *l*-menthol and diethyl ether. Pungent (formic acid) and putrid (hydrogen sulfide) molecules fit because of charge, not shape.

example, in a compound whose molecules contain a small six-carbon-atom benzene ring, shifting the position of a group of atoms attached to the ring may sharply change the odor of the compound, whereas in a compound whose molecules contain a large ring of 14 to 19 members the atoms can be rearranged considerably without altering the odor much. Chemists were led by these facts to speculate on the possibility that the primary factor determining the odor of a substance might be the over-all geometric shape of the molecule rather than any details of its composition or structure.

In 1949 R. W. Moncrieff in Scotland gave form to these ideas by proposing a hypothesis strongly reminiscent of the 2,000-year-old guess of Lucretius. Moncrieff suggested that the olfactory system is composed of receptor cells of a few different types, each representing a distinct "primary" odor, and that odorous molecules produce their effects by fitting closely into "receptor sites" on these cells. His hypothesis is an application of the "lock and key" concept that has proved fruitful in explaining the interaction of enzymes with their substrates, of antibodies with antigens and of deoxyribonucleic acid with the "messenger" ribonucleic acid that presides at the synthesis of protein.

To translate Moncrieff's hypothesis into a practical approach for investigating olfaction, two specific questions had to be answered. What are the "primary odors"? And what is the shape of the receptor site for each one? To try to find answers to these questions, one of us (Amoore, then at the University of Oxford) made an extensive search of the literature of organic chemistry, looking for clues in the chemical characteristics of odorous compounds. His search resulted in the conclusion that there were seven primary odors, and in 1952 his findings were summed up in a stereochemical theory of olfaction that identified the seven odors and gave a detailed description of the size, shape and chemical affinities of the seven corresponding receptor sites.

To identify the primary odors Amoore started with the descriptions of 600 organic compounds noted in the literature as odorous. If the receptor-site hypothesis was correct, the primary odors should be recognized much more frequently than mixed odors made up of two or more primaries. And indeed, in the chemists' descriptions certain odors turned up much more commonly than others. For instance, the descriptions mentioned more than 100 compounds as having a camphor-like odor, whereas only about half a dozen were put in the category characterized by the odor of cedarwood. This suggested that in all likelihood the camphor odor was a primary one. By this test of frequency, and from other considerations, it was possible to select seven odors that stand out as probable primaries. They are: camphoraceous, musky, floral, pepperminty, ethereal (ether-like), pungent and putrid.

From these seven primaries every known odor could be made by mixing them in certain proportions. In this respect the primary odors are like the three primary colors (red, green and blue) and the four primary tastes (sweet, salt, sour and bitter).

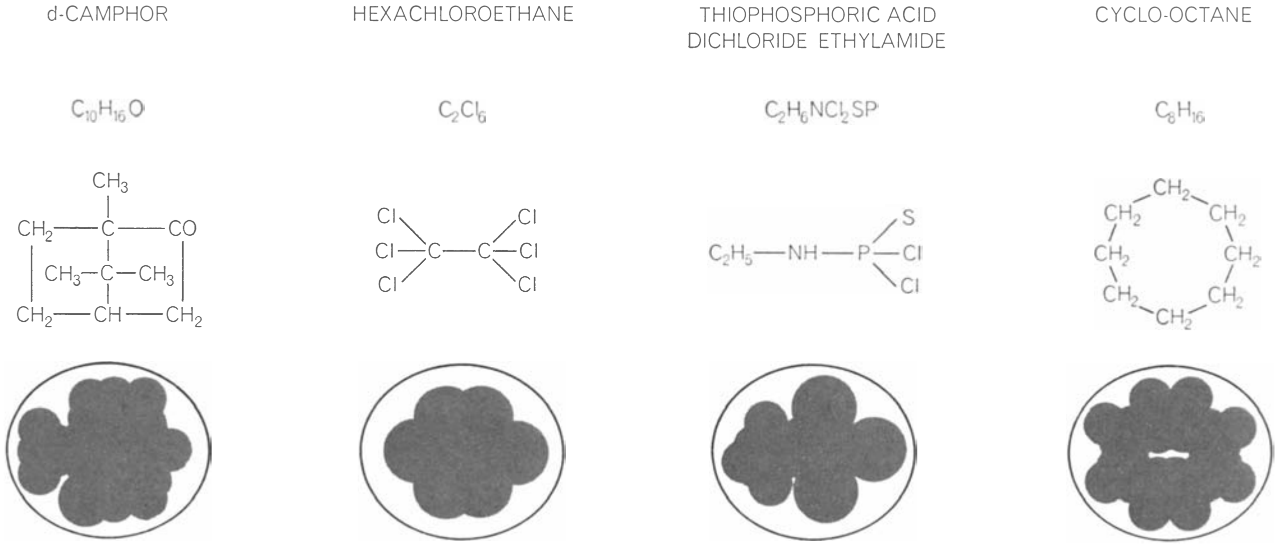
To match the seven primary odors there must be seven different kinds of olfactory receptors in the nose. We can picture the receptor sites as ultramicroscopic slots or hollows in the nerve-fiber membrane, each of a distinctive shape and size. Presumably each will accept a molecule of the appropriate configuration, just as a socket takes a plug. Some molecules may be able to fit into two different sockets—broadside into a wide receptor or end on into a narrow one. In such cases the substance, with its molecules occupying both types of receptor, may indicate a complex odor to the brain.

The next problem was to learn the shapes of the seven receptor sites. This was begun by examining the structural formulas of the camphoraceous compounds and constructing models of their molecules. Thanks to the techniques of modern stereochemistry, which explore the structure of molecules with the aid of X-ray diffraction, infrared spectroscopy, the electron-beam probe and other means, it is possible to build a three-dimensional model of the molecule of any chemical compound once its structural formula is known. There are rules for building these models; also available are building blocks (sets of atomic units) on a scale 100 million times actual size.

As the models of the camphoraceous molecules took form, it soon became clear that they all had about the same shape: they were roughly spherical. Not only that, it turned out that when the models were translated into molecular dimensions, all the molecules also had about the same diameter: approximately seven angstrom units. (An angstrom unit is a ten-millionth of a millimeter.) This meant that the receptor site for camphoraceous molecules must be a hemispherical bowl about seven angstroms in diameter. Many of the camphoraceous molecules are rigid spheres that would inevitably fit into such a bowl; the others are slightly flexible and could easily shape themselves to the bowl.

When other models were built, shapes and sizes of the molecules representing the other primary odors were found [*see illustration on preceding two pages*]. The musky odor is accounted for by molecules with the shape of a disk about 10 angstroms in diameter. The pleasant floral odor is caused by molecules that have the shape of a disk with a flexible tail attached—a shape somewhat like a kite. The cool pepperminty odor is produced by molecules with the shape of a wedge, and with an electrically polarized group of atoms, capable of forming a hydrogen bond, near the point of the wedge. The ethereal odor is due to rod-shaped or other thin molecules. In each of these cases the receptor site in the nerve endings presumably has a shape and size corresponding to those of the molecule.

The pungent and putrid odors seem to be exceptions to the Lucretian scheme of shape-matching. The molecules responsible for these odors are of indifferent shapes and sizes; what matters in their case is the electric charge of the molecule. The pungent class of odors is produced by compounds whose molecules, because of a deficiency of electrons, have a positive charge and a strong affinity for electrons; they are called electrophilic. Putrid odors, on the other hand, are caused by molecules



UNRELATED CHEMICALS with camphor-like odors show no resemblance in empirical formulas and little in structural formulas. **Yet, because the size and shape of their molecules are similar, they all fit the bowl-shaped receptor for camphoraceous molecules.**

that have an excess of electrons and are called nucleophilic, because they are strongly attracted by the nuclei of adjacent atoms.

A theory is useful only if it can be tested in some way by experiment. One of the virtues of the stereochemical theory is that it suggests some very specific and unambiguous tests. It has been subjected to six severe tests of its accuracy so far and has passed each of them decisively.

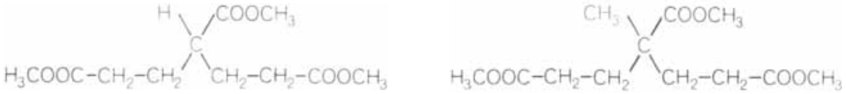
To start with, it is at once obvious that from the shape of a molecule we should be able to predict its odor. Suppose, then, that we synthesize molecules of certain shapes and see whether or not they produce the odors predicted for them.

Consider a molecule consisting of three chains attached to a single carbon atom, with the central atom's fourth bond occupied only by a hydrogen atom [*see top illustration at right*]. This molecule might fit into a kite-shaped site (floral odor), a wedge-shaped site (pepperminty) or, by means of one of its chains, a rod-shaped site (ethereal). The theory predicts that the molecule should therefore have a fruity odor composed of these three primaries. Now suppose we substitute the comparatively bulky methyl group (CH_3) in place of the small hydrogen atom at the fourth bond of the carbon atom. The introduction of a fourth branch will prevent the molecule from fitting so easily into a kite-shaped or wedge-shaped site, but one of the branches should still be able to occupy a rod-shaped site. As a result, the theory predicts, the ether smell should now predominate.

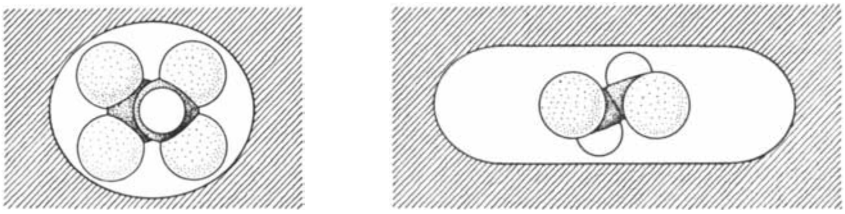
Another of us (Rubin) duly synthesized the two structures in his laboratory at the Georgetown University School of Medicine. The third author (Johnston), also working at the Georgetown School of Medicine, then submitted the products to a panel of trained smellers. He used an instrument called the olfactometer, which by means of valves and controlled air streams delivers carefully measured concentrations of odors, singly or mixed, to the observer. The amount of odorous vapor delivered was measured by gas chromatography. A pair of olfactometers was used, one for each of the two compounds under test, and the observer was asked to sniff alternately from each.

The results verified the predictions. The panel reported that Compound A had a fruity (actually grapelike) odor, and that Compound B, with the methyl group substituted for the hydrogen atom, had a pronounced tinge of the ether-like odor. This experiment, and the theory behind it, make understandable the earlier finding that the odor of certain benzene-ring compounds changes sharply when the position of a group of atoms is shifted. The change in odor is due to the change in the over-all shape of the molecule.

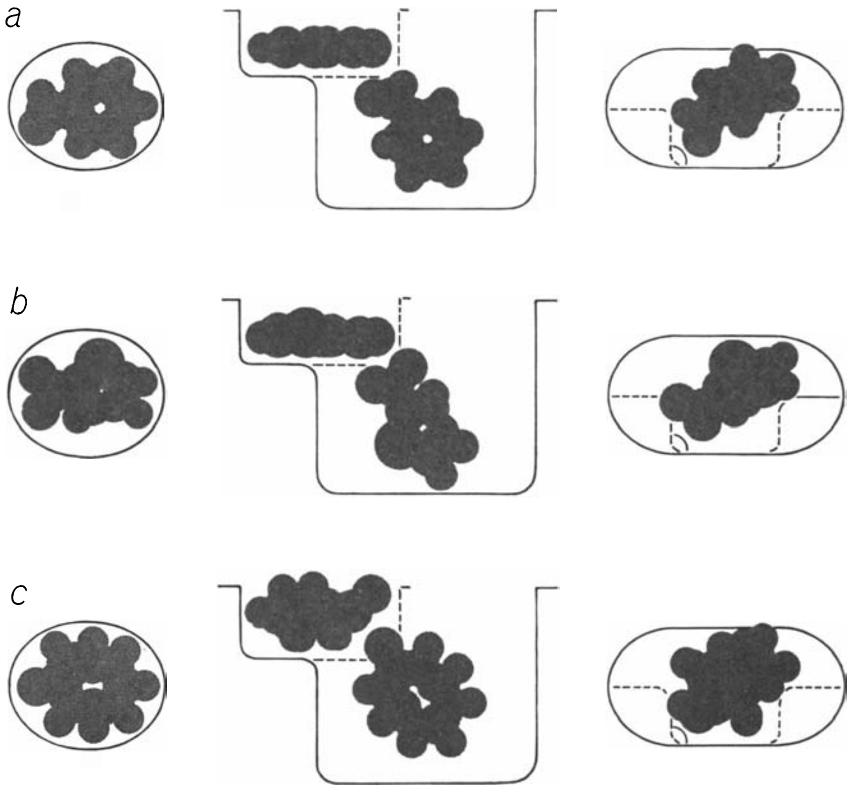
A second test suggested itself. Could a complex odor found in nature be matched by putting together a combination of primary odors? Taking the odor of cedarwood oil as a test case, Amoore found that chemicals known to



CHANGE IN SHAPE of a molecule changed its odor. The molecule at left smelled fruity because it fitted into three sites. When it was modified (*right*) by the substitution of a methyl group for a hydrogen, it smelled somewhat ethereal. Presumably the methyl branch made it fit two of the original sites less well but allowed it still to fit the ethereal slot.



SINGLE CHEMICAL has more than one primary odor if its molecule can fit more than one site. Acetylenetetrabromide, for example, is described as smelling both camphoraceous and ethereal. It turns out that its molecule can fit either site, depending on how it lies.



COMPLEX ODORS are made up of several primaries. Three molecules with an almond odor are illustrated: benzaldehyde (*a*), alpha-nitrothiophen (*b*) and cyclo-octanone (*c*). Each of them fits (*left to right*) camphoraceous, floral (with two molecules) and pepperminty sites.

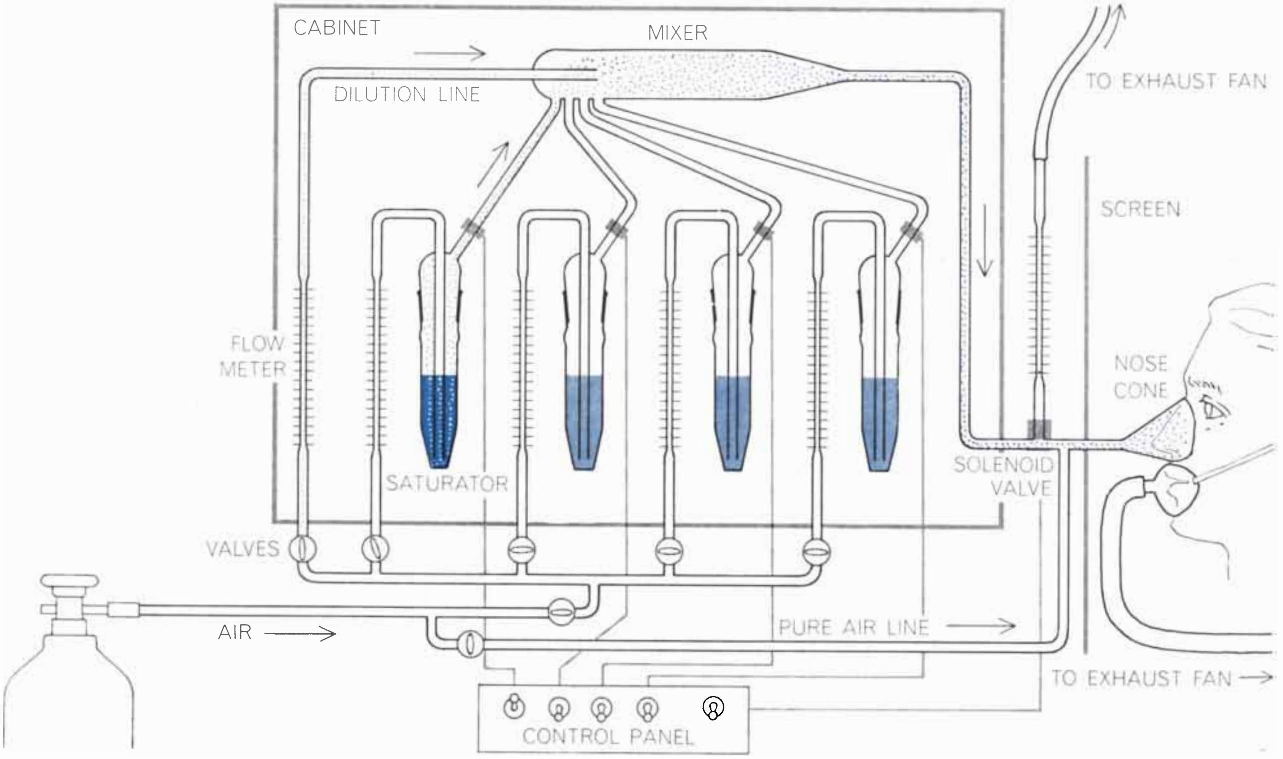
possess this odor had molecular shapes that would fit into the receptor sites for the camphoraceous, musky, floral and pepperminty odors. Johnston proceeded to try various combinations of these four primaries to duplicate the cedarwood odor. He tested each mixture on eight trained observers, who compared the synthetic odor with that of cedarwood oil. After 86 attempts he was able to produce a blend that closely matched the natural cedarwood odor. With the same four primaries he also succeeded in synthesizing a close match for the odor of sandalwood oil.

The next two tests had to do with the identification of pure (that is, primary) odors. If the theory was correct, a molecule that would fit only into a receptor site of a particular shape and size, and no other, should represent a primary odor in pure form. Molecules of the same shape and size should smell very much alike; those of a different primary shape should smell very different. Human subjects were tested on this point. Presented with the odors from a pair of different substances whose molecules nonetheless had the same primary shape (for example, that of the floral odor), the subjects judged the two odors to be highly similar to each other. When the pair of substances presented had the pure molecular traits of different categories (for instance, the kite shape of the floral odor and the nucleophilic charge characteristic of putrid compounds), the subjects found the odors extremely dissimilar.

Johnston went on to make the same sort of test with honeybees. He set up an experiment designed to test their ability to discriminate between two odors, one of which was "right" (associated with sugar sirup) and the other "wrong" (associated with an electric shock). The pair of odors might be in the same primary group or in different primary groups (for example, floral and pepperminty). At pairs of scented vials on a table near the hive, the bees were first conditioned to the fact that one odor of a pair was right and the other was wrong. Then the sirup bait in the vials was replaced with distilled water and freshly deodorized scent vials were substituted for those used during the training period. The visits of the marked bees to the respective vials in search of sirup were counted. It could be assumed that they would tend to visit the odor to which they had been favorably conditioned and to avoid the one that had been associated with electric shock, provided that they could distinguish between the two.

So tested, the honeybees clearly showed that they had difficulty in detecting a difference between two scents within the same primary group (say pepperminty) but were able to distinguish easily between different primaries (pepperminty and floral). In the latter case they almost invariably chose the correct scent without delay. These experiments indicate that the olfactory system of the honeybee, like that of human beings, is based on the stereochemical principle, although the bee's smelling organ is different; it smells not with a nose but with antennae. Apparently the receptor sites on the antennae are differentiated by shape in the same way as those in the human nose.

A fifth test was made with human observers trained in odor discrimination. Suppose they were presented with a number of substances that were very different chemically but whose molecules had about the same over-all shape. Would all these dissimilar compounds smell alike? Five compounds were used for the test. They belonged to three different chemical families differing radically from one another in the internal structure of their molecules but in all five cases had the disk shape characteristic of the molecules of musky-odored substances. The observers, exposed to



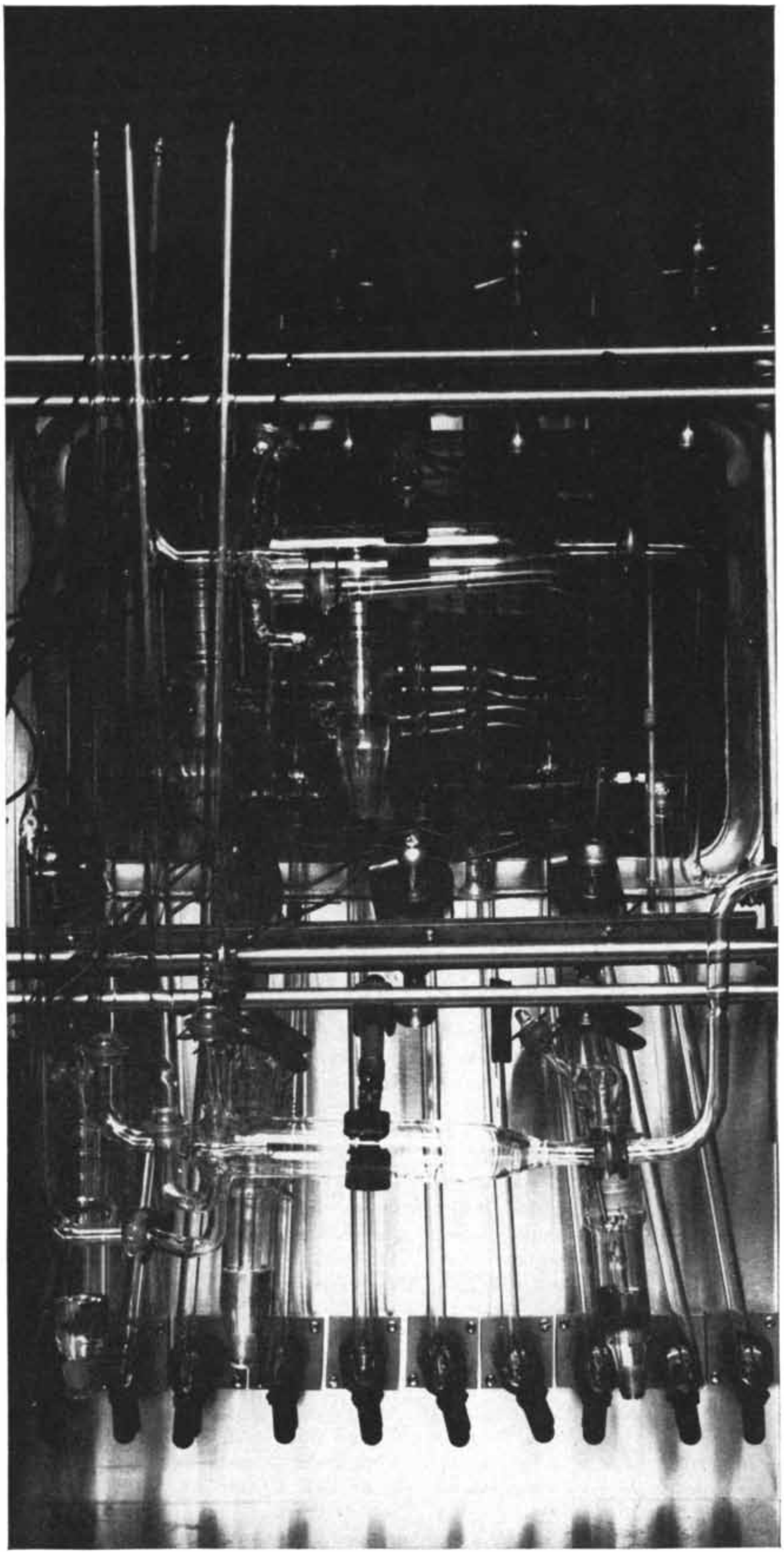
OLFACTOMETER developed by one of the authors (Johnston) mixes odors in precise proportions and delivers them to a nose cone for sampling. This schematic diagram shows the main elements. Air bubbles through a liquid in one of the saturators, picks up odorous molecules and is then diluted with pure air or mixed with air carrying other odors. The experimenter controls the solenoid valves.

the vapors of these five chemicals among many others by means of the olfactometer, did indeed pick out and identify all five as musky. By the odor test, however, they were often unable to distinguish these five quite different chemicals from one another.

Basically all this evidence in favor of the stereochemical theory was more or less indirect. One would like some sort of direct proof of the actual existence of differentiated receptor sites in the smelling organ. Recently R. C. Gesteland, then at the Massachusetts Institute of Technology, searched for such evidence. He devised a way to tap the electric impulses from single olfactory-nerve cells by means of microelectrodes. Applying his electrodes to the olfactory organ of the frog, Gesteland presented various odors to the organ and tapped the olfactory cells one by one to see if they responded with electric impulses. He found that different cells responded selectively to different odors, and his exploration indicated that the frog has about eight such different receptors. What is more, five of these receivers correspond closely to five of the odors (camphoraceous, musky, ethereal, pungent and putrid) identified as primary in the stereochemical theory! This finding, then, can be taken as a sixth and independent confirmation of the theory.

Equipped now with a tested basic theory to guide further research, we can hope for much faster progress in the science of osmics (smell) than has been possible heretofore. This may lead to unexpected benefits for mankind. For man the sense of smell may perhaps have become less essential as a life-and-death organ than it is for lower animals, but we still depend on this sense much more than we realize. One can gain some appreciation of the importance of smell to man by reflecting on how tasteless food becomes when the nose is blocked by a head cold and on how unpleasantly we are affected by a bad odor in drinking water or a closed room. Control of odor is fundamental in our large perfume, tobacco and deodorant industries. No doubt odor also affects our lives in many subtle ways of which we are not aware.

The accelerated research for which the way is now open should make it possible to analyze in fine detail the complex flavors in our food and drink, to get rid of obnoxious odors, to develop new fragrances and eventually to synthesize any odor we wish, whether to defeat pests or to delight the human nose.



CONSTANT-TEMPERATURE CABINET maintains the olfactometer parts at 77 degrees Fahrenheit. The photograph shows the interior of the cabinet, containing two units of the type diagramed on the opposite page. Several of the saturators are visible, as are two mixers (*horizontal glass vessels*), each of them connected by tubing to a nose cone at right.