Table 2. Intracellular distribution of rat-liver glucose-6-phosphatase and arylsulfatase.

Fraction	Percentage of activity of homogenate	
	Glucose-6-phosphatase	Arylsulfatase
Nuclei, washed twice	9.7	9.3
Heavy and light mitochondrial fraction, washed once	12.1	10.9
Microsomes, unwashed	75.4	71.8
Final supernatant	3 2	4.7
Recovery	100.4	96.7
Reconstituted homogenate	87.1	90.6

of 4-nitrocatechol liberated and the amount of enzyme used could be obtained. Roy (*2*) reported similar observations and Maengwyn-Davies and Friedenwald have shown that this nonproportionality is attributable to an endogenous inhibitor, which they have shown to be inorganic phosphate (*10*).

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Olfactometric Method Utilizing Natural Breathing in an Odor-Free "Environment"

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Since 1935, when Elsberg and Levy first described the blast- and stream-injection techniques for measuring olfactory sensitivity in human beings (*1*), their suggestion that prepared stimuli be blown into the nostril(s) under pressure has dominated much experimentation in olfaction. The earlier method of using a sniff has frequently been supplanted by the unnatural one of having the subject suspend his breathing while a substitute "sniff" is blown in. After working with variations of the latter method for some time, I have finally abandoned it for many problems, despite my previous endorsement (*2*). The inability of most subjects to perform reliably, even with long training, was one reason for changing; lack of control over the position of internal mouth and throat parts that affect the volume of air admitted was another; and the extreme artificiality of the situation, which raised the question of generalizing to ordinary breathing, was a third.

No artificial mechanism is as efficient as sniffing in carrying air to the olfactory membrane, and there is no reason to believe that it is necessary to control sniff size if concentration of the gaseous mixture being sniffed is controlled so that the number of odorous molecules available, as well as the volume of inodorous air, can be specified.

In 1921, Zwaardemaker (*3*) described what he called a camera inodorata, an unventilated box of glass and aluminum for use with his olfactometer. The subject, with his head inside the box, sniffed through the olfactometer tube that was inserted into his nostril. Thus, the absolute threshold could be measured in an atmosphere relatively free of uncontrolled odors. A much more elaborate "box," actually two glass rooms called an olfactorium, was described in 1950 by Foster *et al.* (*4*) as providing an odor-free, climatically controlled environment for the whole subject. Although neither of these devices has been put to much use by others, the principle appears sound. Accordingly, I have built a modern camera inodorata, avoiding the tremendous cost and space demands of the olfactorium but still achieving the goal of surrounding the subject's head with continually flowing odor-free air during an experimental session. Instead of using a separate olfactometer to test sensitivity, I simply add controlled amounts of odor to the air in the box; the subject is allowed to sniff at will.

The box, in this case, is made of Plexiglas, and has a top and four walls with an inlet near the upper rear corner of one long wall. Inside dimensions are 45.5 cm long by 35.1 cm wide; the walls are 0.6 cm thick. All inside surfaces are perfectly smooth and entirely Plexiglas, yet the box comes apart completely and easily for cleaning. The bottom is loosely closed with a piece of Pliofilm having a slit down the center to serve as an entrance for the head and an exit for the air. The subject's hair and face (except the nostrils) can be covered with plastic materials to eliminate their odors.

The subject is continuously supplied with pure air at a rate of about 13 ft^3/min, enough to insure that positive pressure always exists inside the box so that other air cannot enter. In an adjoining room, a blower draws room air, previously filtered while coming in from outside, through another filter (*5*) of activated carbon, filterdown, and absolute filter paper (*6*), and propels it into the box through a Plexiglas tube 5.3 cm in diameter that passes through the wall and joins the inlet in the side of the box.

The system for odor production, modified from a previous one (*7*), connects with this system for fresh air supply. Odor control is achieved by the saturation

of a stream of pure air with odor by bubbling the air through odorous liquid, maintenance of the saturated gas at constant pressure and temperature to reach equilibrium, and the release—under very slight pressure—of specified amounts of odorous air whenever desired by means of a combination of valves and an electronic timer. Given these conditions, the number of odorous molecules in any stated volume of gas can be calculated if an acceptable value for the vapor pressure of the odorous compound at 20°C can be determined. The concentration of the stimulus can then be expressed as a stated number of odorous molecules added to a given amount of pure air.

The idea of natural breathing in an atmosphere where amount of odor can be controlled was essentially untried for threshold studies. Experience with it has now shown its success in traditional threshold measurement. Figure 1 shows a curve obtained for one subject for measurement of the difference threshold. The data were collected in three 45-min sessions, with 30 judgments per point, using a modification of the method of single stimuli, as was previously described (*7*). In the standard method, the subject is presented with a number of variable stimuli in random order for judgment concerning intensity without a standard stimulus for comparison. In the present procedure, only one pair of stimuli was used at a time, each pair being equidistant about the same midpoint. The subject was uninformed about the procedure but was given four sample stimuli before each session to illustrate the range of strength he might encounter. About every 30 sec, one member of the pair was added to the pure background air and the subject judged it as "strong" or "weak." If the pair with the largest difference (1.34 and 2.25×10^{17}) is called *A*, the next largest (1.54 and 2.05) *B*, and the smallest (1.64 and 1.94) *C*, the order of the pairs over the 3 days was *AB, CC, BA*. A rest of 1 min, when no odor was added, was given after every five stimuli. Curves of comparable regularity have been obtained in measuring the absolute threshold by the method of limits.

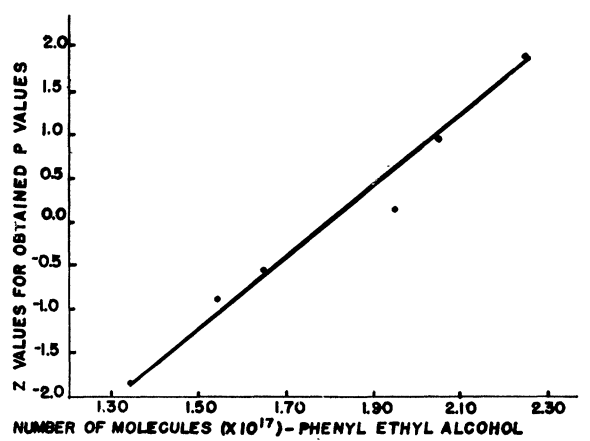

Fig. 1. Relative discrimination data, using phenyl ethyl alcohol and a modified method of single stimuli. The method of plotting transforms the original ogive into a straight line by converting the percentages of "strong" judgments (obtained *p* values) to *z* scores.

The principal advantages of this type of system are (i) the similarity of the subject's task to normal smelling conditions, as contrasted with the extreme artificiality of the blast injection method; (ii) the ease with which subjects take to the task, which requires no special training; (iii) control of the environment around the subject's head, the only body region directly involved in study of olfactory sensitivity; and (iv) the feasibility of using standard psychophysical procedures since each stimulus is quickly removed by means of the continuous flow of pure air. The method is worthy of consideration for use in studies of thresholds, adaptation, mixtures, and a number of other problems, using any human subject who can understand the simple task, and even using reasonably small animals that can be trained to give an indicator response (*8*).

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Bio-oxygenation of Progesterone by Mushrooms

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In recent years the ability of filamentous fungi to form hydroxyl functions on various steroids and at differing carbon positions of these molecules has been a subject of considerable interest. Details of work involving studies on the class Phycomycetes and the class Fungi Imperfecti have been well summarized by Peterson (*1*).

In this light it became of interest to determine whether a similar enzymatic mechanism could be observed to function among members of the class Basidiomycetes. For this study we have cultured various mushroom species under submerged fermentation conditions, as were first described by Humfeld (*2*) and later extended by Humfeld and Sugihara (*3*). Preliminary results of these studies with some of the various mushroom species are presented here.

Conventional fermentation procedures using a ro-