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.

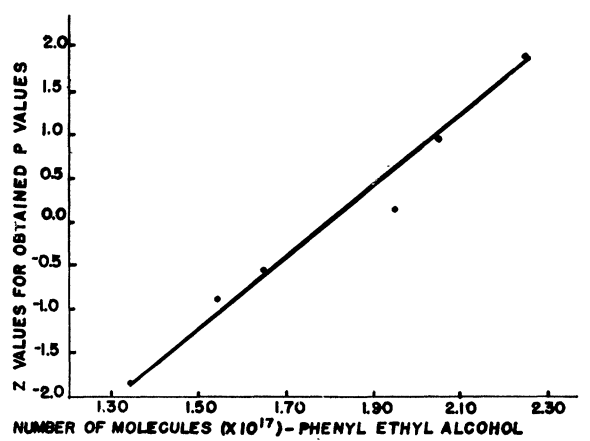

Fig. 1. Relative discrimination data, using phenyl ethyl alcohol and a modified method of single stimuli. The method of plotting transforms the original ogive into a straight line by converting the percentages of "strong" judgments (obtained *p* values) to *z* scores.

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-

Table 1. Biomodifications of progesterone by various mushroom species.

Mushroom species	Culture *	Filter-paper chromatography† R_T‡								Infrared spectral modifications of progesterone§
Agaricus campestris	NRRL2334		0.15	0.25	0.41		0.69		2.5	Strong —OH, other changes at 6.1 and 9.0μ regions
Agaricus rodmanii	M21									Weak —OH, $\overset{O}{\overset{\parallel}{C}}$ at 5.8μ, other changes at 6.1μ
Lepiota naucina	NRRL2368		0.12							Weak —OH
Lepiota procera	M44									Weak —OH, other changes not now identified
Lepiota rachodes	M76	0.04	0.12					1.7		Strong —OH
Pleurotus ostreatus	NRRL2366		0.15			0.50	0.72	1.6		Strong —OH
Cantharellus cibarius	NRRL2370								2.2	Moderate —OH
Armillaria mellea	M6a		0.19		0.38					Strong —OH, new bands 5.8–6.0μ, changes at 6.2μ
Hebeloma sinapizans	M84		0.19							Strong —OH
Tricholoma nudum	NRRL2371		0.16						2.0, 2.4	Strong —OH, other changes at 9.0μ
Lycoperdon umbrinum	NRRL2372		0.10		0.37				2.4	Weak —OH
Morchella crassipes	NRRL2369								2.1	Weak —OH

* M-numbered cultures were kindly supplied by T. F. Sugihara, Western Utilization Research Branch, Albany, Calif. NRRL cultures were obtained through the courtesy of C. W. Hesseltine, Northern Utilization Research Branch, Peoria, Ill.

† Resolved in propylene glycol-toluene, 18 hr at 25°C, spots were detected by ultraviolet scanning, and 2,4,DNPH spray reagent. In some instances small amounts of unconverted progesterone were observed using the solvent systems described by Bush (7).

‡ Rate of movement of steroid/rate of movement of compound "S."

§ CH_2Cl_2 extracts of fermentation beers.

tary shaker unit were employed for the growth of the mushroom mycelia and for the steroid conversion studies. In general, the conditions employed for the conversions paralleled those described by Murray *et al.* (*4*). In the present work, 30 to 50 mg of crystalline progesterone dissolved in 1.0 ml of methanol was added to the growing mushroom mycelia (48-hr growth) cultivated in 250-ml erlenmeyer flasks containing 50 ml of nutrient broth and 2 percent glucose. After an additional 24 hr of growth, the culture beers were filtered on Whatman No. 1 paper and both the beer filtrate and the mycelia were extracted three times with methylene chloride and were pooled. The solvent was then evaporated to dryness with a warm air blower followed by vacuum desiccation. The recovered steroid residues were then freed of fatty materials by a Girard separation procedure and were examined by paper-chromatographic methods (*5*) in order to detect the formation of oxygenated products. Further examination of the recovered steroid products by means of infrared spectrometry was performed (*6*). For this study preparations were dissolved in chloroform at 10-percent concentration and were examined in a Baird double-beam spectrometer equipped with a sodium chloride prism.

The data obtained from the paper-chromatographic and infrared-spectral interpretations are presented in Table 1. It is interesting to observe that, although compounds with similar mobilities are present in the fermentation extracts of several different species, in no instance is the pattern of spots identical. Likewise, it is evident that exposure of the progesterone substrate to the growing mushoom cultures results in the production of a variety of steroidal components with greatly decreased paper-chromatographic mobilities, consistent with the oxygenation of progesterone.

When the fermentation extracts were examined by infrared spectrometry, a large number of the spectra showed the presence of a band at the 3-μ region indicative of the formation of hydroxyl functions in the steroid extracts. A control specimen of heat-killed mycelia failed to provide any changes in the absorption spectrum of progesterone when it was treated under similar conditions.

From the data provided it is evident that the fungi of the class Basidiomycetes can readily perform oxygenation and other biomodifications of steroids. A more detailed description of the isolation and characterization of some of the steroid products is being prepared and will be submitted for publication elsewhere.

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