of absorption is obtained and the cancer is produced sooner and with a smaller total dose. With pellets a total of five to ten milligrams is sufficient, whereas with injections in oil a total of 30 to 40 milligrams is used.

In a parallel group of experiments, an attempt has been made to induce mammary carcinoma of the rat with other estrogenic substances. The compounds used have been estradiol, estradiol dipropionate and diethylstilboestrol. The experiments are still incomplete but cancer has been obtained in one rat injected with 200 gamma of diethyl-stilboestrol daily for 100 days. This cancer induced with diethyl-stilboestrol is significant because the substance has the physiological action of estrone but is not a sterol. This suggests that the mammary cancers induced in the rats by the injection of estrogenic substances are due to the physiological changes produced rather than to the cancerogenic nature of the chemicals used.

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## FUMARIC ACID FORMATION ASSOCIATED WITH SEXUALITY IN A STRAIN OF RHIZOPUS NIGRICANS

A DEFINITE relation has been known for some time to exist between the sexuality of fungi belonging to the *Mucorales* and certain biochemical reactions exhibited by them. Thus Satina and Blakeslee[1] demonstrated a high degree of correlation of the sexual nature of these fungi with such properties as reducing power of cell extracts toward $KMnO_4$ and tellurium salts, Manoilov's reaction, catalase content, etc. On the other hand, these authors could not establish any differentiation based on the carbohydrase systems of the various races. The differences in reactions obtained for the male and female races were in almost all cases quantitative rather than qualitative.

The present note concerns one strain of *Rhizopus nigricans* whose male and female races are distinct by virtue of the *possession or the complete lack of a certain specific physiological mechanism,* namely, the enzyme system by which the organism produces fumaric acid when grown on a glucose-mineral solution (Table I). So far as the authors are aware no similar case has yet been reported. The male race (−) was tested under a variety of experimental conditions and never was found to form fumaric acid, whereas the female race (+) produced this acid abundantly. Further, despite the fact that a large part of the glucose consumed was transformed by the plus race into fumaric acid, this organism was able to utilize the energy much more efficiently and synthesize much more cell substance than its male homologue, as measured by nitrogen consumption.

The ability to form fumaric acid is but one of the many specific reactions of which this group of fungi

TABLE I

FUMARIC ACID PRODUCTION BY MALE (−) AND FEMALE (+) RACES OF A STRAIN OF RHIZOPUS NIGRICANS*

| | Female race (+) | Male race (−) |
|---|---|---|
| Glucose consumed, gm. | 6.472 | 6.662 |
| Fumaric acid produced, gm. | 2.059 | 0 |
| Conversion, per cent. | 31.8 | 0 |
| $NH_3$–N consumed in culture, mgm. | 80.7 | 40.6 |
| Calcium in solution due to organic acids, mgm. | 722.1 | 49.6 |
| Fumaric acid equivalent, calculated from $Ca^{++}$, gm. | 2.094 | ..... |
| Per cent. of total acid as fumaric | 98.3 | ..... |

* Medium used: 200 cc portions of 5 per cent. glucose-mineral solution containing 82.0 mgm $NH_3$–N; incubation, 15 days at 28° C.

is capable, and it is not impossible that sexual differences within this group may eventually be correlated with other as yet unstudied physiological processes. It must not be inferred, however, that the above results are characteristic of all or even a significant number of sexual pairs of *Rhizopus;* another pair tested simultaneously yielded no such dissimilarity and even members of the same race differed in this respect. This would seem to indicate that fumaric acid formation is not at all a property specific to a race but may rather be attributed to some biological characteristic giving rise to strain specificity.

A detailed study of the process of fumaric acid production and its possible function in the mechanism of energy utilization by different species of *Rhizopus* will be published shortly.[2]

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## GAS BUBBLES AS NUCLEI FOR "OOLITES"[1]

SPHERICAL bodies of calcium carbonate, with some of the characteristics of oolites, are now forming in a swimming pool supplied with water from one of the Pinkerton Hot Springs in southwestern Colorado. Five large springs and several smaller ones occur within an area of about a quarter of a square mile on the west side of the Animas River, about 12½ miles north of Durango. They appear to issue from fissures in the Ouray limestone, but the points of emergence are obscured by cones of travertine that have been deposited by the spring waters. The year-round tem-

[1] S. Satina and A. F. Blakeslee, *Proc. Nat. Acad. Sci.*, 12: 191–96, 197–202, 1926; 13: 115–22, 1927; 14: 308–16, 1928.

[2] The authors are indebted to Dr. A. F. Blakeslee for supplying cultures used in these investigations.

[1] Published by permission of the Director, Geological Survey, United States Department of the Interior.