is related to adrenal and/or hypophyseal function. Removal of these glands abolishes tachyphylaxis, but the adrenalectomized, hydrocortisone-treated rat does develop tachyphylaxis. Therefore, this physiological compensatory mechanism is mediated by adrenal and/or hypophyseal hormones. This suggestion is consistent with the findings of Goodman and Knobil(2) who found that the pituitary and adrenal cortex are not necessary for lipid mobilization during fasting, but the lipolytic response is reduced following ablation of these glands and cortisol administration to adrenalectomized rats returned the lipolytic response to normal. The postulated role of the adrenal and pituitary glands in development of tachyphylaxis to U-19425 is further supported by Pereira's findings in nicotinic acid-treated, adrenalectomized, hypophysectomized rats(11). He found that the characteristic plasma FFA rebound to levels higher than normal after nicotinic acid did not occur if both glands were removed, but FFA rebound above normal if only one or the other was removed.

Summary. After 4 days of treatment with U-19425, rats no longer respond to the drug with a decrease in plasma FFA and blood sugar 2 hours after treatment. The initial plasma FFA response to the drug is similar, but pretreated rats escape from antilipolytic effects of U-19425 faster than nonpretreated controls. Tachyphylaxis to 5-methylpyrazole-3-carboxylic acid may be due to a physiological compensatory mechanism which is mediated by the adrenal and/or pituitary glands.

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Intracellular Localization of 3-4 Benzo(a)Pyrene in *Saccharomyces cerevisiae.* (32496)

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The yeast, *Saccharomyces cerevisiae,* was used as a test cell to investigate the intracellular localization of Benzo(a)pyrene. The localization was investigated primarily by ultraviolet microscopy, and the observations were correlated with intravital staining with Janus Green B and other techniques.

Materials and methods. Saccharomyces cerevisiae (strain 7921 American Type Culture Collection) was cultivated and tested in Middlebrook 7H9 broth base with 5% (w/v) Dextrose.

Organisms were grown aerobically in the dark at 35°C in glass 20 × 150 mm screw capped tubes.

Benzo(a)pyrene (Aldrich Chemical Co., Milwaukee, Wis.) was purified in a darkened room by paper chromatography on Whatman No. 1 filter paper using solvents prepared by the method of Lijinsky and Raha (1). Spectral characteristics of the purified eluants were identical with standards of pure Benzo(a)pyrene prepared in this laboratory. A stock solution containing 2 mg

of 3-4 Benzo(a)pyrene per ml of acetone were stored in dark brown glass bottles at 12°C in complete darkness. All experiments were performed in a darkened room using glassware and other equipment opaqued by metal foil.

A concentration of 2×10^{-6} g/ml 3-4 Benzo(a)pyrene in the culture medium was used in all experiments and prepared by aspirating 0.01 ml of stock solution into a Hamilton microliter syringe and forcibly expelling the contents directly into 10 ml of medium while manually agitating the culture tube. Observation of "labeled" media prepared in this was at 100× magnification under the ultraviolet microscope showed no particulate fluorescent material until 18-24 hours after addition of the Benzo(a)pyrene stock solution.

Ultraviolet microscopic examinations were made of wet smears using achromatic objectives, a 200 watt mercury vapor light source and Zeiss No. 1 excitor and No. 47 barrier filters. The method of Doan and Ralph(2) using Janus Green B was employed for intravital staining.

Results. When 24-hour cultures are observed under the ultraviolet microscope at 450× magnification, no fluorescence is detectable in the cells until approximately 3 hours after addition of Benzo(a)pyrene to the medium. Intracellular fluorescent globules rapidly appear at this time and essentially all cells are fluorescent after 5 hours (Fig. 1). Older cultures "stain" more rapidly. Seven-day-old cultures show fluorescent cells after 2 hours exposure.

In the cells, fluorescent particles are distributed in a characteristic circular fashion toward the peripheral portion of the cell. The globular intracellular particles are relatively uniform in size measuring approximately 0.5-0.75 μ in diameter. The location of the particles within the cell and their size is remarkably constant. The majority of cells contain 12-20 such fluorescent globules. The cell wall, cell nucleus, central vacuole, and capsule of the organism are not fluorescent. Cultures older than 14 days contain some cells exhibiting fluorescence of central vacuole

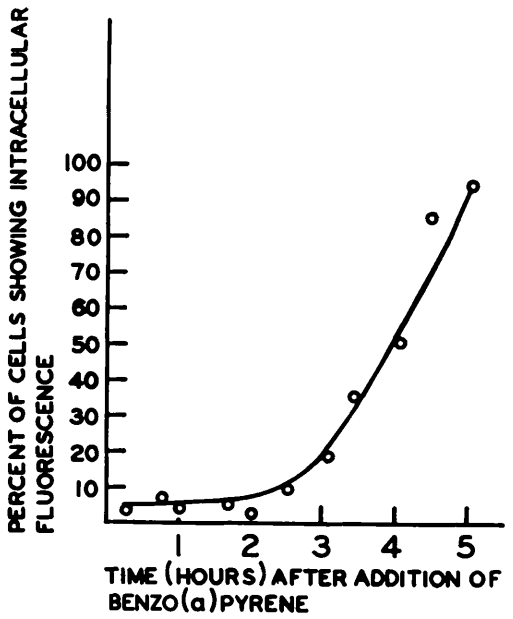

FIG. 1. Uptake of Benzo(a)pyrene by *Saccharomyces cerevisiae* in 24 hr synthetic broth culture as measured by percentage of total cells exhibiting intracellular fluorescent bodies.

or vacuoles and the fluorescent granules are more variable in size.

When fluorescent cells are compared with intravital stained preparations of cells, grown under identical conditions, Janus Green stained bodies of similar size and location are noted in the cytoplasm of the cell between the central vacuole and the cell membrane (Fig. 2 & 3). Methanol fixed smears stained with Oil Red O in supersaturated isopropanol(3) and by the method of Grogg and Pearce(4) for acid phosphatase showed no lipidic or acid phosphatase stainable bodies in these cells.

Discussion. The morphologic observations presented suggest that Benzo(a)pyrene or its derivatives, is bound to mitochondria in *Saccharomyces cerevisiae.* Both the fluorescent and intravital stained bodies correspond closely in number, size, and location to electron microscopic observations of mitochondria in aerobic cultures of *Saccharomyces cerevisiae*(5). Neither our data, nor those of others(6) provides convincing morphologic evidence of intraction of Benzo(a)pyrene with nuclear structures in yeast.

Conclusions. Vegetative cells of *Saccharo-*

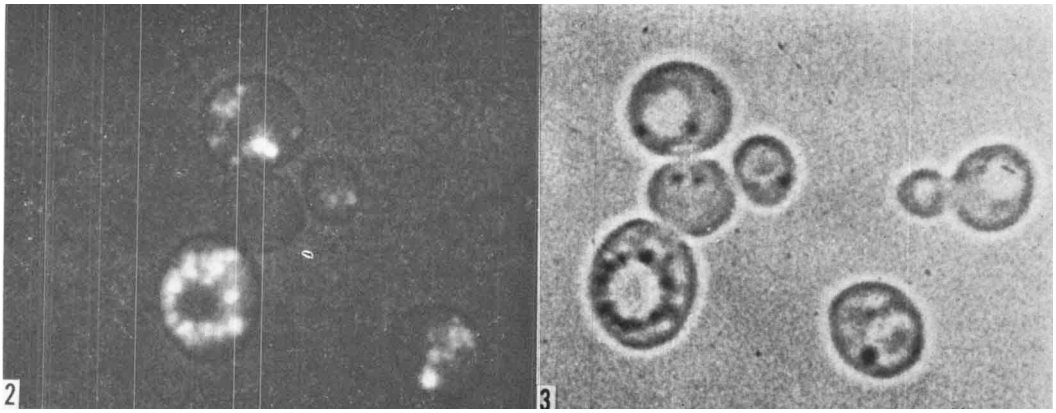

FIG. 2. 24 hr aerobic culture of *Saccharomyces cerevisiae* cells, 4 hours after addition of 2.0×10^{-6}g/ml Benzo(a)pyrene to the medium. Ultraviolet transmitted light 1200 ×. Fluorescent bodies are present in 4 cells, one of which shows circular arrangement of globules.

FIG. 3. Field identical to that depicted in Fig. 2 intravital Janus Green B stain. Tungsten light; 1200 ×. The distribution of stained bodies is almost identical to that seen in the fluorescent preparation.

myces cerevisiae incubated with 3-4 Benoz-(a)pyrene at a concentration of 2×10^{-6} g/ml during the logarithmic phase of cell growth in a synthetic medium show localization of fluorescence to intracytoplasmic organelles tentatively identified as mitochondria on the basis of comparison with Janus Green B stained cells and with electron micrographs. Other cellular structures were not fluorescent.

Summary. Vegetative cells of the common yeast *Saccharomyces cerevisiae* grown in a synthetic medium were incubated in the presence of 2×10^{-6} g/ml of Benzo(a)pyrene. Ultraviolet microscopic studies of cells in the logarithmic growth phase show isolated fluorescence of intracytoplasmic organelles which appear to be mitochondria on the basis of comparison with intravitally stained cells.

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