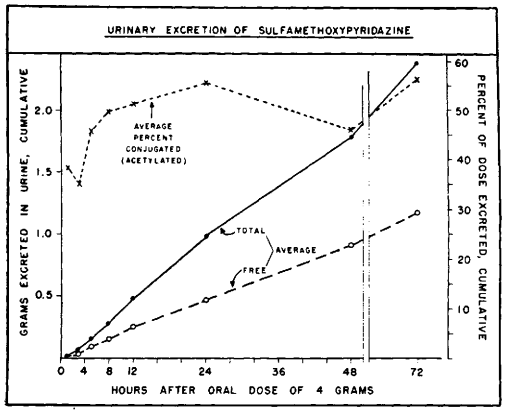

FIG. 4.

jects. Specimens of urine collected 105 hours after the dose still contained concentrations of drug averaging 19.3 mg % total and 7.8 mg % free.

Untoward effects. The urinary sediment was examined microscopically in specimens collected between 8 and 24 hours and revealed no abnormalities. The only untoward effect experienced by 3 of the 6 subjects was some lassitude followed by frontal headache that began between 3 and 5 hours after the dose and lasted 4 or 5 hours; the headache was aggravated by sudden motions of the head but this did not interfere with normal activity.

Summary and conclusions. Concentrations of sulfamethoxypyridazine in blood and urine of 6 normal adult males were determined after a single oral dose of 4 g. The drug was well absorbed, yielding high levels of free drug and only small amounts in acetylated form in the plasma. Little if any of the drug diffuses into the blood cells. The drug is cleared slowly from the plasma, the acetylated form being cleared by the kidney about 11 times as fast as the free drug. Urine concentrations varied up to about 200 mg %, between 35 and 60% being in the conjugated form. Significant levels were still present in the blood and urine 105 hours after the dose. This prolonged action should be of clinical interest and suggests that further exploration of the potentialities of this new sulfonamide drug is warranted.

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Morphological Transformation of *Candida albicans* in Tissues of Mice.* (22570)

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Organisms of the genus *Candida* exhibiting filamentous morphology are commonly seen in smears prepared from the oral and vaginal cavities of infected persons. Cells of *C. albicans* with filamentous morphology have also been observed in deeper tissues including nervous tissue, kidneys, adrenals, cardiac and skeletal muscle and various portions of the gastrointestinal tract(1-5).

In rabbits experimentaly infected by the intravenous injection of *C. albicans,* large numbers of filamentous cells are found especially in kidneys(6,7). On the basis of morphology in tissue Baker(8) has classified *C. albicans* with those fungi which are found in tissue as "filaments and round bodies." Scherr and Weaver(9) have speculated that "when the mycelial phase of the fungus becomes evident in the later stages of an infection, it would appear that some factor which normally favors the Y (yeast) forms has diminished in function."

While studying the host tissue reaction to washed yeast-phase cells of pathogenic fungi, it was observed that cells of *C. albicans* rap-

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idly underwent morphological alterations after injection into the subcutaneous tissues of mice. The nonpathogenic members of the genus *Candida* studied did not exhibit this change. The morphological alteration of *C. albicans* was so rapid as to suggest a possible role in the pathogenesis of experimental moniliasis in the mouse.

Materials and methods. Six different strains of *C. albicans,* all virulent for mice, and one strain each of *C. stellatoidea, C. tropicalis, C. pseudotropicalis, C. krusei, C. parapsilosis* and *C. guilliermondi* were used in this study. Two of the strains of *C. albicans* were of recent origin, one isolated from the oral cavity of a 5-year-old child with a clinical diagnosis of oral thrush and the other from the throat of a week-old infant exhibiting white, membranous plaques in the oral cavity. The remaining strains of *C. albicans* had been maintained on Sabouraud medium for undetermined periods of time. All strains of *C. albicans* and the single strain of *C. stellatoidea* readily produced chlamydospores on the glycogen medium described by Nickerson and Mankowski(10).

The organisms were maintained on Sabouraud-maltose agar with yeast-extract (0.01%). All of the strains grew in the typical smooth, yeast-like phase on this medium. The inoculum for injection into mice consisted of organisms washed with 0.85% sodium chloride solution from 48 hour agar cultures incubated at 35°C. The cells were washed 2 times by centrifugation and resuspended in fresh saline solution. No attempt was made to standardize the number of organisms contained in the suspensions other than by visual approximation.

Adult albino mice were used in these experiments. Cells of members of the genus *Candida* were injected into the subcutaneous tissues of these animals and the inflamed areas were studied at various time intervals after injection. One-tenth ml of the above suspensions of cells was injected into the subcutis of the animal's abdomen. At different time intervals mice were sacrificed and thin sheets of loose connective tissue were excised from within the area of injection. These sheets of areolar tissue were rapidly spread on microscope slides, air-dried and stained by the May-Grünwald-Giemsa method. The yeast cells stained a light blue by this procedure which made them easily distinguishable from host cells. This technic was essentially that used by Schneebeli and Dougherty(11) to study early inflammation in the mouse.

A second method similar to the first was used later because of greater ease in preparing the connective tissue spreads(12). It was a modification of the "granuloma pouch" technic of Selye(13). One ml of air was injected subcutaneously into the nape of the neck of mice. Subsequently the organisms contained in 0.2 ml of diluent were injected into the formed air pouch. At different times after inoculation animals were sacrificed and the intact air pouch partially dissected free. Sheets of connective tissue comprising the wall of the base of the pouch were removed and treated as previously described.

Results. A striking morphological alteration occurred in the cells of all of the strains of *C. albicans* studied. Within 60 minutes after injection into the subcutaneous tissues of mice almost all of the yeast-like cells of *C. albicans* had formed a short rudimentary pseudomycelium (Fig. 1a). Cells suspended in the saline diluent retained their typical yeast-like morphology. Various stages in development of the pseudomycelia were observed. Slides prepared 2 hours after injection revealed an increase in length of the pseudomycelia with beginning septa formation (Fig. 1b). Four hours after injection definite septa were evident (Fig. 1c). With the exception of *C. stellatoidea* these *in vivo* alterations in morphology were not observed when other species of *Candida* were injected. These organisms retained their typical, yeast-like morphology (*e.g.* Fig. 1e and 1f). The single strain of *C. stellatoidea* which was studied exhibited *in vivo* pseudomycelial formation similar to that shown by *C. albicans* strains.

At 6, 12 and 24 hours after injection of yeast-like cells of *C. albicans* and *C. stellatoidea* typical pseudomycelia were observed in spreads prepared from the loose connective tissues of mice. In the case of *C. albicans* some were observed which were several oil-

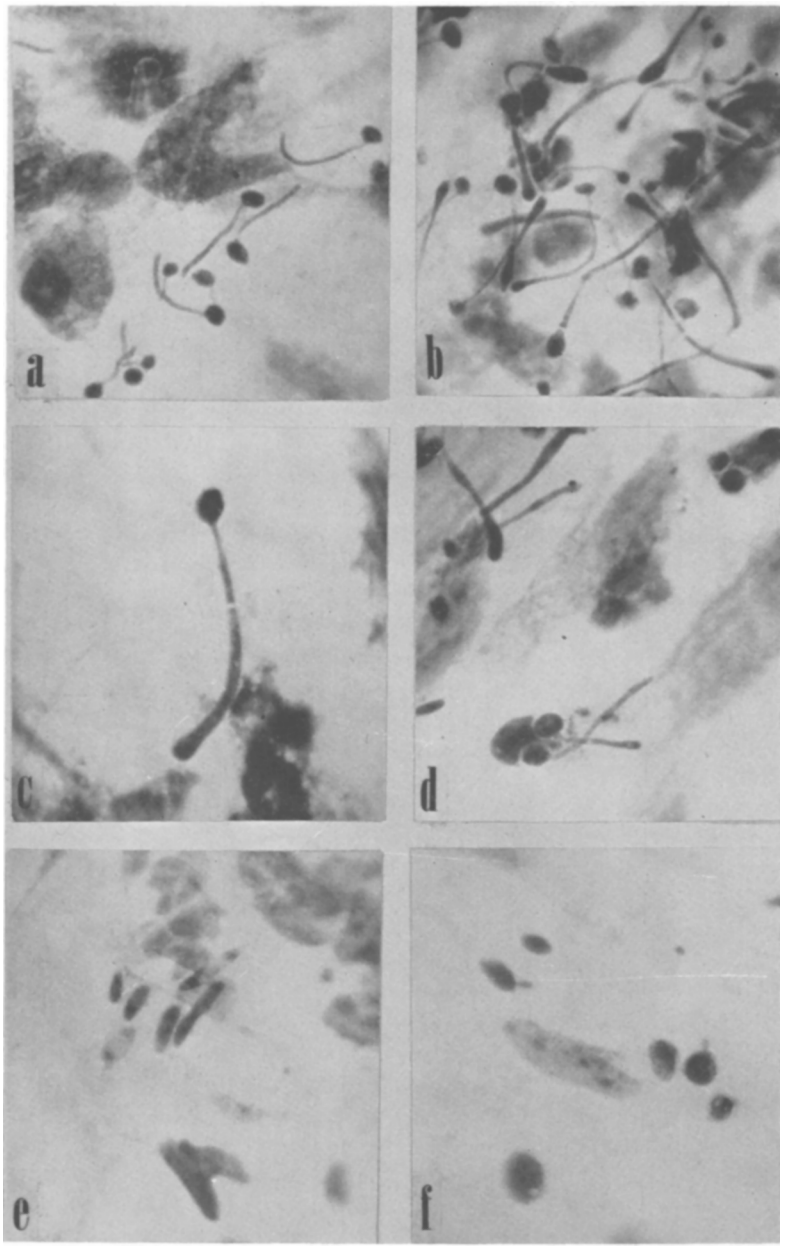

FIG. 1. Appearance of cells of *C. albicans* and other *Candida* species after inj. of washed yeast-like cells into the subcut. tissues of mice. a. *C. albicans* 1 hr after inj. b. *C. albicans* 2 hr after inj. c. *C. albicans* 4 hr after inj. d. *C. albicans* cells 2 hr after inj. demonstrating ingestion by phagocytes. e. *C. krusei* 4 hr after inj. f. *C. tropicalis* 4 hr after inj.

immersion fields in length. Lateral blastospores were observed. As judged by their staining characteristics, however, the majority of the organisms observed 24 hours after injection appeared to be degenerating. In the case of other species of *Candida* studied it was difficult to demonstrate organisms in the subcutaneous connective tissues of mice at this latter time. Phagocytosis of yeast-like cells was observed in subcutaneous tissues taken from mice injected with all species of *Candida* studied. The ingested cells exhibited

various stages of digestion as evidenced by loss of the ability to take the basophilic stain. With preparations from mice injected with cells of *C. albicans* rarely was a phagocyte observed which had ingested an organism showing any degree of pseudomycelium formation (Fig. 1d). Occasionally a phagocyte was seen which had ingested the "head" portion with the "tail" projecting outward.

Discussion. After completion of these observations the work of Stovall and Pessin(7) was found in the literature. They studied the behavior of injected cells of *"Monilia albicans"* (*C. albicans*), *"M. candida"* (*C. tropicalis*) and *"M. parapsilosis"* (*C. parapsilosis*) in rabbits. It was reported that only the cells of *"M. albicans"* would form pseudomycelia *in vivo*. The authors suggested that *"M. albicans"* produced lesions by a "purely mechanical plugging of capillaries and arterioles." However, on the basis of measurements of cells grown for 48 hours on malt agar, they were unable to find a correlation between cell size and pathogenicity of the 3 organisms studied. The principal quality which accounted for the virulence of *"M. albicans"* was suggested to be the ability to grow in the animal body. It is apparent that the size of yeast-like cells of *C. albicans* grown on malt agar has little relationship to the size of the filamentous cells *in vivo*. The findings reported here partially substantiate the earlier work of Stovall and Pessin(7). Of the organisms studied in this laboratory, only *C. albicans* and *C. stellatoidea* exhibited filamentous morphology in the tissues of the mouse. All species of *Candida* usually considered non-pathogenic failed to develop pseudomycelia *in vivo*. The fact that the strain of *C. stellatoidea* behaved similarly to the strains of *C. albicans* would appear to indicate their close relationship. The rapidity of the alterations in morphology of *C. albicans* in the connective tissues of the mouse would seem to indicate a significant role for this alteration in the pathogenesis of experimental moniliasis. It would appear reasonable to suggest that this transformation favors survival of the fungus in the host by a mechanical interference of ingestion by phagocytes and by possible "mechanical plugging of capillaries and arterioles." On the basis of the speculation that the yeast-like form of *C. albicans* is best adapted to development in tissues, Scherr (14) postulated that any agent which would transform the organism from the yeast-like phase to the mycelial phase *in vivo* might arrest the multiplication of the pathogen. To examine this hypothesis he tried a number of compounds with plant growth-promoting activity. These compounds were given to mice systemically infected with *C. albicans*. The results of these experiments were negative. The fact that large numbers of filamentous organisms are found in the tissues of infected mice and rabbits may indicate that exactly the opposite situation may exist, *i.e.*, that the filamentous form of *C. albicans* is best adapted to growth in animal tissues. Experiments designed to test this suggestion are in progress. Factors affecting the morphology of *C. albicans in vivo* are also under investigation.

Summary. A morphological alteration of yeast-like cells of *C. albicans* and *C. stellatoidea* has been shown to occur within 1 hour after injection into the subcutaneous tissues of mice. Yeast-like cells of other members of the genus *Candida* failed to exhibit these alterations under the same conditions. The yeast-like cells of *C. albicans* had formed elongated pseudomycelia within 1 hour after injection. At later times considerable growth of these filaments with the appearance of septa was observed. Other species of the genus *Candida* retained their typical yeast-like morphology. It has been postulated that filamentation of *C. albicans in vivo* serves as a hindrance to ingestion by mouse phagocytes. The results suggest a significant role for these morphologically altered organisms in the pathogenesis of experimental moniliasis in the mouse.

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Pathophysiology of Malignancy: I. Tissue Oxygen Tension of Benign and Malignant Tumors of the Skin.* (22571)

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Many facets of tumor metabolism have been investigated, leading to the conclusion that demonstrable alterations of aerobic and anaerobic respiration exist in malignant cells (1,2). Yet little attention has been paid to the physiologic aspects of oxygen transport and supply in tumor tissue. Thus it seemed appropriate to investigate the parameters of tumor tissue oxygen tension, and study the mechanisms of its control.

In the past, tissue oxygen tension has been mainly estimated from the oxygen content of arterial and venous blood. This method, limited to organs whose rate of capillary circulation can be measured accurately, leads only to gross estimates of tissue oxygen tension changes, and is generally unsuitable for most malignant tumors because of the difficulty of obtaining representative samples of venous blood. Before the introduction of the platinum oxygen cathode, the nearest approach to a satisfactory method for measuring oxygen tension in intact tissues was the one in which gas bubbles, introduced into a tissue, were allowed to come to equilibrium in respect to oxygen, and the gas then withdrawn and analyzed(3). This method is prone to large errors due to compression of surrounding vessels, it cannot measure rapid changes of pO_2 nor is it applicable to estimation of oxygen tension of small volumes of tissue.

The platinum oxygen cathode, described by Davies and Brink(4), has been used extensively for rapid estimation of oxygen tension in intact skin(5), heart(6) and muscle(7). A description of the basic instrumentation and the physiologic variables applicable to its use in skin has been given by Montgomery and Horvitz(5).

Method. Cutaneous oxygen tension was measured *in vivo* by means of the open type oxygen cathode(5). The diameter of the exposed platinum surface was approximately 200 μ. In order to minimize variations in blood flow, all measurements were performed in a warm room (temperature 28° to 30°C) and comparative pO_2 determinations of abnormal and adjacent normal skin were performed simultaneously. The electrodes used were calibrated against each other in saline (equilibrated against air) to be certain that the platinum surfaces exposed were comparable. Three or more consecutive values (obtained by re-insertion of the electrodes into adjacent tumor and normal skin sites respectively) were averaged and compared to each

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