tain conditions the inhibition by α-methylglutamic acid could be overcome. A possible explanation was suggested for this finding and the importance of taking readings at several time intervals was emphasized.

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## Virulence Enhancing Activities of Aureomycin on *Candida albicans.** (19418)

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Personal observations and a number of reports in the literature(1) indicate that proliferation of fungus-like organisms, notably *Candida albicans* may occur in patients treated with penicillin, aureomycin, chloramphenicol. and terramycin. Not only is there a numerical increase in the oral cavity, intestinal or urogenital tract, etc., but localized or disseminated disease with serious, and at times fatal consequences may develop.

The mechanism of stimulation of growth and increase in pathogenicity of fungi in patients treated with antibiotics is not fully understood. The frequency of these complications has become of sufficient impact to warrant the American Council on Pharmacy and Chemistry of the A.M.A. to issue "a warning statement to be included in aureomycin hydrochloride, chloramphenicol and terramycin hydrochloride labeling"(2). The Council suggests that susceptible bacteria are suppressed by the bacteriostatic action of the drugs, and monilia or other yeast-like organisms may then replace the normal or abnormal bacterial flora, thus paving the way for secondary mycotic infection. The following experiments may shed some light on these problems.

*Material and methods. Candida albicans.* A strain isolated from the oral cavity of a patient was used for all the tests. The strain grew in fluid media with sediment and no pellicle formation, fermented dextrose, maltose and sucrose, did not attack lactose. On Sabouraud's agar it produced white, creamy colonies, on blood agar plates dull grey colony forms and on corn meal agar streaks, it developed the characteristic mycelium and yeast cell clusters. It was therefore considered a typical *Candida albicans*. For animal tests, the growth from Sabouraud's agar slants was washed off with normal saline solution and

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centrifuged at 1500 r/min. The white sediment of packed cells was suspended in saline (0.1 ml cells + 2.4 ml saline). 0.1 ml of the cell sediment contained approximately 4 billion cells. The usual dose of 0.2 ml of the suspension corresponded to 320 million living cells. The animal injections were given intraperitoneally. *Aureomycin.* "Aureomycin crystalline intravenous Lederle", was used. The brown crystals come in sealed ampules containing 100 mg of the drug. 10 ml normal saline were introduced into the ampule and the contents dissolved. 1 ml of the yellow solution corresponds to 10 mg aureomycin, the dose of 0.2 ml normally used to 2 mg. Because of the known lack of stability of these solutions,† only freshly prepared ones were employed unless stated otherwise.

*Experiments.* The strain of *Candida* employed proved to be nonpathogenic for mice on intraperitoneal injection of large amounts of cells (320 million) in saline. Of 18 mice so treated all survived after 2 weeks. The aureomycin dose of 2 mg intraperitoneally was non-toxic. Although the animals became restive for 1-2 minutes after the obviously painful injection of the latter, all of the 17 mice so treated survived after 2 weeks. However when *Candida* cells were suspended in aureomycin solution (0.1 ml cells + 2.4 ml aureomycin) and the mixture injected intraperitoneally, 16/17 mice died. The following Table I (A-C) demonstrates the combined results of 3 experiments.

On 32 repeated administrations of the mixture, 28/32 mice died in 3-48 hours. No gross signs of peritonitis or involvement of organs (kidney, spleen, liver, adrenals) were seen. In each case, *Candida* was recovered on culture from the peritoneal cavity, and in 10 out of 27 on heart blood culture. In 3 mice there was complication of *S. enteritidis* infection and these animals were excluded from the evaluation. Numerous *Candida* strains recovered from dead mice were tested again in normal mice. None had acquired pathogenicity. When the aureomycin-*Candida* mixtures were centrifuged,

† On standing, precipitation begins on the second day and considerable sediment is present in 3-4 days.

TABLE I. Action of Candida, Aureomycin, and of Their Mixture in Mice.

| Mice group | Material injected, .2 ml | Mortality* |
|---|---|---|
| A | Candida cells in saline sol. | 17/17 |
| B | Aureomycin sol. | 17/17 |
| C | Mixture of Candida cells in aureomycin | 1/17 |
| D | Candida cells in aureomycin susp. | 0/9 |
| E | Supernate of Candida-aureomycin mixture after centrifugation | 9/9 |
| F | Washed Candida sediment | 8/9 |

* Numerator: Survivors after 2 wk. Denominator: Total No. of mice.

TABLE II. Separate Injection of Aureomycin and Candida Cells in Mice.

| Mice group | Treatment | Results* |
|---|---|---|
| A | Inj. 24 hr *before* infection | 5/9 |
| B | 8 | 4/9 |
| C | 2 | 1/10 |
| D | Simultaneous inj. and infection | 1/10 |
| E | Inj. 4 hr *after* infection | 3/10 |
| F | 8 | 9/10 |
| G | 24 | 10/10 |

* Numerator: Survivors after 2 wk. Denominator: Total No. of mice.

Inj = i.p. injection of 2 mg aureomycin sol., infection = i.p. inoculation of .2 ml Candida suspension in saline.

and the supernatant and washed sediment injected separately in amounts corresponding to 0.2 cc of the original mixture, they proved non-toxic, Table I (D-F). There was thus no indication of an interaction between aureomycin and *Candida* organisms *in vitro.* In another group of experiments, aureomycin and *Candida* organisms were administered separately at different time intervals.

From Table II it is apparent that when aureomycin is given from 24 hours before until 4 hours after infection with *Candida* there was a considerable mortality; the toll was higher as the aureomycin injection and *Candida* infection were administered at closer intervals. If, however, *Candida* cells were subjected to action of the mouse for 8 or 24 hours before administration of aureomycin, the increased pathogenicity did not occur. One may conclude that under these conditions the antifungal activities of the mouse tissues could play their part, while with reversed order of the injections aureomy-

cin interfered for a considerable time with the normal defense mechanism of the animal.

It is known that aureomycin solutions kept at icebox or room temperature for a few days lose considerable antibiotic potency. When such solutions were used in the *Candida* tests above reported, the virulence enhancing capacity was also lost. Ten mice were injected intraperitoneally with 0.2 ml each of a suspension of 0.1 ml packed cells in 2.5 ml aureomycin solution which has been kept at room temperature for two days: all 10 survived.

The antibiotic capacity of aureomycin in solution is heat labile. When the solution of crystals in saline was brought to the boiling point and then used for the "*Candida*-aureomycin mixture test", 13 of 14 mice injected intraperitoneally with 0.2 ml of the mixture survived. It is apparent therefore, that a parallelism exists between antibiotic (against bacteria) and virulence enhancing (for *Candida*) faculties of aureomycin solutions.

*Discussion.* The problem of fungus proliferation *in vivo* under antibiotic influence is not only one of survival in favorable conditions but also one of activation. It can not be explained by the suppression of sensitive bacteria alone and consequent lack of competition for nutritive substances or by induced suppression of growth-restraining factors dependent upon the elimination of the customary bacterial flora. Direct growth-promoting action of the antibiotics as well as changes of inherent pathogenicity of the fungi might be involved. Most antibiotics are known to promote growth of animals(3). That the effect is not necessarily due to control of the intestinal flora was stressed by Weber *et al.*(4). Elam and co-workers(5) reported that parenteral application of penicillin and bacitracin while not influencing the fecal flora at all was able to promote growth in the chick.

Although some workers have found a direct growth-promoting action of antibiotics on fungus cells *in vitro*, the results in other instances have been inconclusive or negative (6-9). In our own experiments, aureomycin in ordinary and synthetic liquid media or on nutrient agar slants has failed to promote the growth of *Candida albicans*. The possibility remains that the host's tissues undergo biological changes under the influence of antibiotics and then respond to *Candida* infections (6,10-13). Indications of lowered resistance due to the action of aureomycin were seen in our experiments. If the animal's normal defense mechanism against *Candida* was allowed to act for 8-24 hours, the subsequent injection of aureomycin was almost entirely without harm. If the order of injections was reversed, the preceding aureomycin treatment interfered with the defensive reaction of the mouse. Infection 8 or 24 hours later shows still enhanced virulence of *Candida*.

Lowering of resistance against infections has been revealed as the result of mucin action in animals(14,15) and as an effect of cortisone (16,17). Aureomycin may well join this group of biological activators as illustrated in its mode of action against *Candida*.

*Summary.* 1. *Candida* suspensions are nonpathogenic, and aureomycin solutions nontoxic, when either of these alone is injected intraperitoneally into mice. The mixture of both is fatal. 2. A similar "drug-fungus" effect though less pronounced was observed when aureomycin was given from 24 hours before to 4 hours after *Candida* inoculation. Given 8 and 24 hours after infection aureomycin was ineffective. 3. The virulence enhancing activity of aureomycin was destroyed on standing at room temperature or by boiling, a parallel to its antibiotic activity. 4. No indication of toxin production or biological alteration of *Candida* was found *in vitro*. 5. No direct growth stimulating effect by aureomycin on *Candida* was observed in cultures. The action *in vivo* was one of lowering the animal's resistance.

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## Reduced Antibody Titers in Mice Treated with Carcinogenic and Cancer Chemotherapeutic Agents. (19419)

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It has long been known that x-radiation, in addition to inhibiting(1) and initiating(2) neoplastic growth, depresses antibody levels in experimental animals(3). More recently this triad of effects has been demonstrated for nitrogen mustard(4-6). Further it has been reported that the Rous sarcoma virus in addition to initiating tumors will also depress antibody levels(7). Chemotherapeutic substances which have recently been shown to inhibit both tumor growth and antibody production are A-methopterin(8), benzene(9), cortisone (10) and 8-azaguanine(11).

In view of the foregoing observations the following work was undertaken to determine whether or not antibody inhibition is a property common to other substances involved in tumor production and inhibition.

*Materials and methods.* The data are based chiefly on results obtained in homozygous strain C (B alb C) mice 2 to 6 months old. However, the tests were frequently repeated in homozygous strain C3H mice also 2 to 6 months old, and the same results were obtained. The antibody forming mechanism was evaluated by determining sheep cell hemolysin titers of mouse serum harvested 5 days after the intraperitoneal injection of 0.5 cc of a 4% suspension of washed sheep cells in saline. The hemolysin titers were determined by noting the degree of hemolysis resulting from 30 minute incubation at 37°C of 0.5 cc of serial 2-fold dilutions of test sera with 1.0 cc of 1:20 guinea pig serum and 0.5 cc of 1% sheep cells. Carcinogenic compounds and their non-carcinogenic analogues from three major groups of carcinogens were studied. These included 1) methylcholanthrene, 1,2,5,6-dibenzanthracene, 1,2-benzanthracene‡ and phenanthrene,§ 2) butter yellow (*p*-dimethylaminoazobenzene) and *p*-diethylaminoazobenzene,§ 3) urethan (ethyl carbamate), isopropyl carbamate,‡ *β*-chloroethylcarbamate,§ and methyl carbamate.§‖ Test sub-

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† National Institutes of Health, Federal Security Agency.

‡ An analogue with weak carcinogenic activity.

§ Non-carcinogenic analogue.

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