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The Effect of Antibiotics on the Growth and Survival of Candida Albicans in the Intestinal Tract of Mice

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THE EFFECT OF ANTIBIOTICS ON THE GROWTH AND SURVIVAL OF CANDIDA ALBICANS IN THE INTESTINAL TRACT OF MICE

by

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LIFE

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CHAPTER I

INTRODUCTION

Soon after broad spectrum antibiotic therapy(Aureomycin,Neomycin, Streptomycin,etc.)attained widespread clinical use,reports of secondary monilial(*Candida albicans*)infections of the mouth,alimentary canal,and lungs, began to appear in the literature(Bartels and Buchbinder,1945;Harris,1950; Williams,1950;Moore,1951;Woods,Manning and Patterson,1951;Smith,1952; Kligman,1952;Bratlund and Holten,1954;Loh and Baker,1955;Schaberg et al., 1955). Such secondary infections may vary from a relatively mild(Sharp,1954) to an extremely severe and possibly fatal condition(Gausewitz et al.,1951; Rankin,1953;Brown et al.,1953;Zimmerman,1955;Levy and Cohen,1955). Various theories have been offered in explanation for the mechanism which brings about the increased susceptibility to infection by *C. albicans* during antibiotic therapy.

Miller(1950)suggested suppression with substitution as the mechanism. He felt that secondary infection by *C. albicans* resulted from elimination by the antibiotic therapy of the normal bacterial flora which tends to maintain a state of equilibrium. He described this restraining action of the population as bacterial antagonism.

In 1951 Woods,Manning and Patterson reported they were unable to correlate clinical observations of secondary infection by C. albicans following penicillin, aureomycin, and chloramphenicol therapy with in vitro experiments designed to show direct stimulation of the fungus by these antibiotics. From this study they concluded that suppression of growth of bacteria and other organisms normally competing with C. albicans for nutritional substances may be the most important factor in the overgrowth of C. albicans following the use of antibiotics. However in their in vitro studies the concentration of aureomycin used was only 0.1 mg./ml. Other workers(Huppert et al.,1953) reported that a stimulatory effect can be demonstrated in vitro only at higher concentrations of the antibiotic.

The studies of Moore(1951) and Pappenfort and Schnall(1951) are also in conflict with the in vitro results reported by Woods, Manning and Patterson. Moore(1951) reported that the growth of C. albicans in broth containing crystaline aureomycin hydrochloride appeared to be twice as great as in similar cultures without antibiotic. In addition, he reported that the cells on the bottom of the flask in close proximity to undissolved crystal of the antibiotic were considerably larger than normal, and that cells in the veil of growth on the surface of the broth were markedly smaller than normal. Both of these observations were interpreted as evidence for stimulation of the growth of C. albicans by aureomycin. Pappenfort and Schnall(1951)measured the effect of aureomycin on C. albicans using the diffusion plate method. C. albicans was suspended in Sabourauds glucose agar in a petri dish, and a solution of aureomycin was placed in cups in the agar. A zone of increased growth was noted in the area of diffusion around the cups. The same effect was obtained with six different lots of aureomycin prepared for oral administration. Huppert, MacPherson and Cazin(1953) confirmed Moore's observations that the growth of C. albicans in broth containing aureomycin was twice as great as in similar cultures without antibiotic. They reported that C. albicans grown in broth containing aureomycin yielded 2.1 mg./ml. nitrogen as compared to 1.0 mg./ml. nitrogen in cultures without the antibiotic.

Huppert and Cazin(1955a) demonstrated that chlortetracycline, neomycin, and bacitracin stimulate the growth of C. albicans in vitro while penicillin, streptomycin, magnamycin, chloramphenicol, oxytetracycline, erythromycin, and tetracycline do not. However, all of these antibiotics had been associated with secondary infections of C. albicans. The authors concluded from these observations that there is no apparent correlation between direct stimulation of C. albicans under in vitro conditions and the developement of secondary fungus infection during antibiotic therapy.

It has also been suggested that changes in the pH of the intestinal menstruum, as well as the presumably higher concentration of nutrient material present in the intestinal tract after suppression of the normal bacterial flora, may be responsible for the increased susceptibility to C. albicans infection(Foley and Winter, 1949; Woods et al., 1951). Karnaky(1946) demonstrated that C. albicans grew equally well over a pH range of 3.9 to 10.8. It thus seems unlikely that a change in the pH of the intestinal menstruum would appreciably affect the growth of C. albicans, but it is possible a change in the pH of the intestinal menstruum may affect other organisms in the intestinal tract and thus indirectly also C. albicans. Burkholder(1943) showed that C. albicans grew on a synthetic medium composed of glucose and ammonium salts, and required only the addition of biotin for maximal growth. Since C. albicans has very minimal growth requirements, it seems doubtful whether destruction of the intestinal flora would add to the environment any nutrients required for growth of C. albicans, which are not present in the normal host.

Harris(1950) felt that the mucous membrane complications(oral, vaginal, rectal, and intestinal) following aureomycin therapy were attributable to a vitamin B complex deficiency resulting from the elimination of the intestinal bacteria which may be normally involved in the synthesis of these vitamins. When a potent vitamin B complex preparation was administered parenterally to patients receiving aureomycin, he found a reduction in incidence and severity of the mucous membrane lesions. As Harris points out, however, the vitamin treatment was studied in only eleven patients. Furthermore, since the lesions develop rapidly after the onset of antibiotic therapy, avitaminosis is probably not a causative factor. In spite of these considerations, he felt that suppression of the intestinal flora would allow the overgrowth of C. albicans which could then invade tissues whose resistance had been lowered by a vitamin B complex deficiency. As yet there appears to be no direct evidence to substantiate the assumption of avitaminosis as the mechanism by which the secondary fungus infection occurs. We do know that antibiotics alter the flora of the intestinal tract and vitamin synthesis (Anderson et al., 1953a, 1953b). But what contribution this synthesis makes to the total vitamin supply of the host has not been established. Bierman and Jawetz(1951)reported experiments on the effect of prolonged administration of multiple antibiotics on the human fecal flora. They stated that no nutritional deficiency was noted during therapy in patients with adequate food intake.

Henrici(1941)suggested that C. albicans may elaborate a toxin responsible for its pathogenicity. He based this suggestion on the observation that some infections with C. albicans are clinically similar to infections by Aspergillus which has been shown to produce a toxin(Henrici,1941).

Winner(1956)reported the first experimental evidence of toxin production by C. albicans. He found rabbits to possess a considerable degree of natural resistance to intravenous injection of living virulent C. albicans. The presence of natural or induced agglutinins did not protect the animals from lethal doses of the organism,and similar lesions were produced in immunized and non-immunized rabbits. From these observations he suggested that the pathogenicity of C. albicans may involve a toxin.

More recently Roth and Murphy(1957)reported extraction from C. albicans of a factor which was lethal for mice treated with chlortetracycline. This factor was nontoxic in the absence of antibiotic. They regarded the factor as an endotoxin. It would probably be better to regard this factor as a substance which increases the toxicity of the antibiotic rather than an endotoxin since it is inactive when given alone.

Lipnik,Kligman,and Strauss(1952)and Kligman(1952)in a study of fungus infections occuring in conjunction with antibiotic treatment were unable to observe either a potentiating effect of aureomycin,chloromycetin, terramycin, and penicillin on the *in vitro* growth of *C. albicans*, or any evidence of enhancement of systemic infection. In addition they reported that broad spectrum antibiotics caused mucous membrane irritation. Kligman (1952) from these observations postulates that it is the mucous membrane irritation caused by the antibiotic and not a direct action of the antibiotic on the fungus which predisposes to infection. He regarded the infectivity of *C. albicans* as negligible for normal human beings.

Hunter and Foley (1956) found that aureomycin, or cortisone acetate therapy, increased the virulence of *C. albicans* in mice as evidenced by the developement of the characteristic renal lesions following injections of small inocula which were ineffective in untreated mice. Similar results were obtained by Pinkerton and Patterson (1957).

In summary, several possible theories may be advanced to account for the occurrence of secondary monilial infections during antibiotic therapy.

1. Suppression of growth of bacteria and other organisms which in the normal host are competing with *C. albicans* for nutritional substances (Miller, 1951; Robinson, 1954; Woods et al., 1951). This theory has been discussed as a possible mechanism in reviews by McCoy (1954) and Jawetz (1956). Jawetz states, "The suppression of the normal flora is undoubtedly the most important feature in the frequently observed superinfections with *Monilia (Candida)*".
2. The direct stimulation of growth of *Candida albicans* by the antibiotic (Moore, 1951; Pappenfort and Schnall, 1951; Huppert et al.,

1953,1955;Nickerson,1953;McCoy,1954;Eagle and Saz,1955;Jawetz, 1956).

3. A change in the pH of the environment through alteration of the bacterial flora and also the increased concentration of nutrient material following destruction of the normal intestinal flora may play a part in superinfections with C. albicans(Foley and Winter,1949).

4. The normal flora may supply certain required nutrients(eg. vitamins)to the host which may be necessary to maintain resistance to C. albicans infection(Harris,1950). Nickerson(1953) and Jawetz(1956)discuss this as a possible mechanism in their reviews. Nickerson states,"Avitaminosis resulting from suppression of the bacterial flora of the intestine has been viewed as the major predisposing factor to Candida infection."

5. C. albicans may produce a substance which is either toxic as such or which enhances the toxicity of the antibiotic(Henrici, 1941;Roth and Murphy,1957).

6. Antibiotic therapy may cause tissue alterations which lower the resistance of the tissues and make them more susceptible to C. albicans(Winter and Foley,1949;Kligman,1952).

Huppert,Cazin,and Smith(1955b)reported experiments which suggested that mice can be used to simulate the overgrowth of C. albicans in the intestinal tract during oral administration of some antibiotics,particularly aureomycin,as it is found in human beings. These animals might therefore serve as a model for testing some of the theories proposed to explain the occurrence of monilial infections during antibiotic therapy.

The investigations to be reported here were undertaken with a twofold purpose: (1) to investigate to what extent mice can be used to simulate the susceptibility to and the overgrowth of C. albicans brought about by certain antibiotics in man; and (2) to study the mechanism or mechanisms by which antibiotics may increase the susceptibility of mice to superinfection with C. albicans.

CHAPTER II

MATERIALS AND METHODS

One strain of albino mice, Rockland Farms strain RAP, the same as used by Huppert et al., (1955b), was employed in most experiments of this study. A few experiments were carried out with Swiss mice obtained from Abrams Small Stock Breeders, Chicago. Both dealers state that their animals are maintained on a diet which is entirely free of antibiotics. Only mice, weighing 12 to 14 grams or 25 to 30 grams, respectively, were used. The animals were fed Rockland Mouse Diet which is certified to be free of antibiotics. No difference was found in the reaction of the two breeds of mice used.

Candida albicans strain CDC, obtained from the Department of Bacteriology, University of Illinois, was used throughout the study. This strain was maintained by monthly transfer on Sabourauds maltose agar, and stored in the refrigerator.

The two strains of Escherichia coli used in this study were strain 5 and 25. These strains were chosen to test the antagonism of intestinal bacteria to enteric infections by C. albicans because they had been found to be highly antagonistic to experimental enteric infections with Shigella (Freter, R., 1956; Hentges, D., 1957). E. coli strain 5 was isolated in Mexico City from the stool of a 15 month old infant in the summer of 1955. It was made resistant to 1 mg./ml. streptomycin by the gradient plate technique. E. coli strain 25 was obtained from a normal human being and is described by Freter(1956). Both strains were maintained by monthly transfer on veal infusion agar containing 1 mg./ml. streptomycin, and were stored in the refrigerator.

The suspension of C. albicans used for infecting the animals was prepared by inoculating the surface of Chapmans modification of Sabourauds maltose agar and incubating at 37 C for 24 hours. The resulting growth was washed off with 5.0 ml. of 0.85% saline and the suspension standardized by using the turbidity:cell count relationship described below(figure 3).

The E. coli suspension used for introducing this organism into the intestinal tract was prepared by inoculating the surface of veal infusion agar plates containing 1 mg./ml. streptomycin and incubating at 37 C for 24 hours. The resulting growth was washed off with 5.0 ml. of 0.85% saline and the suspension standardized by using the turbidity:cell count relationship described below(figures 1 and 2).

The turbidity:cell count relationships illustrated in figures 1,2, and 3 were determined with cell suspensions prepared by the same procedure as used in the preparation of inocula for animal infections. Serial twofold dilutions were made from the original suspension and the density of each dilution determined with the Klett photoelectric colorimeter using the blue filter number 42. The number of viable cells was then determined by culturing suitable dilutions of the original suspension of each organism. C. albicans suspensions(which consisted of single cells)were spread on the surface of Chapmans modification of Sabourauds maltose agar. Surface plate counts of

E. coli suspensions were carried out on veal infusion agar containing 1 mg./ml. streptomycin. Plates with C. albicans were incubated for 48 hours, those with E. coli for 24 hours at 37 C. The concentration of viable cells in the original suspensions was then calculated from the number of colonies of C. albicans or E. coli on these plates.

Solutions of streptomycin sulfate were made up in sterile tap water. Solutions of aureomycin hydrochloride were prepared by dissolving one capsule (250 mg.) in 100 ml. of sterile tap water containing 1 ml. concentrated hydrochloric acid, specific gravity 1.1895. The solution was then adjusted to pH 5.5 with 0.1 N sodium hydroxide and diluted to the desired concentration with sterile tap water. This procedure was necessary because aureomycin is not soluble at neutrality. It is soluble but unstable at alkaline pH and both stable and soluble at acid pH. The antibiotic solutions were stored in the refrigerator and used only if less than one week old.

The stomach tube used for intragastric inoculations was constructed by attaching a 5 mm. piece of size P.E. 100(ID=.34", OD=.060") polyethylene tubing (Clay-Adams Co. Inc., New York) to the tip of a 20 gauge hypodermic needle.

Three methods were employed for determining the susceptibility of mice to C. albicans. In procedure A, graded inocula of C. albicans were given and the 50% infective dose determined by testing for the presence of Candida in the stools. Mice treated according to procedure B received a standard inoculum of C. albicans. The number of C. albicans in the stool was then determined by quantitative plate counts. Tests for antagonism of E. coli to C. albicans(procedure C)consisted of giving graded inocula of C. albicans plus a standard inoculum of E. coli. The 50% infective dose of Candida was then determined as in procedure A.

Procedure A:

One hundred and twenty mice of specified weight and sex were divided into 4 major groups(groups 1,2,3,and 4). Each of the four major groups was further divided into 5 subgroups of 6 animals each(group 1a,1b,.... etc.). The mice of group 1 received 1 ml. of a 2 mg./ml. solution of aureomycin by stomach tube on day 1. Beginning with day 2 until termination of the experiment, a solution of 1 mg./ml. aureomycin was supplied as drinking water. The mice of group 2 were given 1 ml. of a solution of 0.8 mg./ml. of streptomycin by stomach tube on day 1,and a solution of 0.4 mg./ml. of streptomycin as drinking water on day 2 until termination of the experiment. The mice of group 3 received 1 ml. of a solution containing 2 mg./ml. aureomycin and 0.8 mg./ml. streptomycin by stomach tube on day 1 and a solution of 1 mg./ml. of aureomycin and 0.4 mg./ml. of streptomycin as drinking water on day 2 until termination of the experiment. The mice of group 4 served aw antibiotic free controls and were supplied with sterile tap water for the duration of the experiment. On day 3 the animals of all groups were injected intragastrically with graded inocula of C. albicans suspended in 1 ml. of 0.85% saline. At 2 and 5 days postinfection,each mouse was induced to pass one stool pellet directly into a sterile test tube. Each stool sample was emulsified in 1.0 ml. of 0.85% saline and 0.1 ml. of the resulting suspension was spread on a plate of Chapmans modification of Sabourauds agar. After 48 hours incubation at 37 C,

C. albicans was identified by its characteristic morphology on these plates. C. albicans produced "off white",circular,smooth,convex to pulvinate colonies about 4 mm. in diameter,which were never found in stool cultures from non-infected animals. The 50% infective dose(ID50)of Candida was then determined according to the method of Reed and Muench(1938).

Procedure B:

These experiments involved quantitative determinations of C. albicans in the stool. Forty mice of specified weight and sex were divided into 4 groups of 10 mice each. The treatment of groups 1,2,3,and 4 on days 1 and 2 was the same as that described in procedure A. On day 3 the animals of all groups received a standard inocula(stated in the table of results for each individual experiment)of C. albicans suspended in 1 ml. of 0.85% saline. At 2 and 5 days postinfection,stool samples were taken as described in procedure A. Each stool sample was emulsified in 1 ml. of 0.85% saline. The suspension thus obtained was designated a 1:10 dilution. It contained only single Candida cells and not filaments,as determined by staining and microscopic observation. From the 1:10 dilution further serial ten fold dilutions were made. One tenth ml. of each dilution was spread with a sterile bent glass rod on the surface of Chapmans modification of Sabourauds maltose agar. After 48 hrs. incubation at 37 C counts of C. albicans colonies were made on a quebec colony counter.

Procedure C:

Tests for antagonism between E. coli and C. albicans were carried out as follows: 120 mice of specified weight and sex were divided into 4 major groups(groups 1,2,3,and 4). Each of the 4 major groups was further divided into 5 groups of 6 animals each(group 1a,1b,....etc.). The mice of group 1,2,and 3 received by stomach tube 1 ml. of a 50 ug./ml. solution of streptomycin on day 1 and a solution of 0.4 mg./ml. of streptomycin as drinking water until termination of the experiment. On day 2 the mice of group 1 and 2 received by stomach tube 1 ml. of a suspension of streptomycin resistant E. coli cells suspended in veal infusion broth containing 50 mg./ml. calcium carbonate and 1 mg./ml. streptomycin. On day 2 the mice of group 3 received by stomach tube 1 ml. of sterile veal infusion broth containing 50 mg./ml. calcium carbomate and 1 mg./ml. streptomycin. Thus group 3 served as E. coli free controls. The mice of group 4 were antibiotic free controls and were supplied with sterile tap water until termination of the experiment. On day 3 the animals of group 1 and 2 received by stomach tube graded inocula of C. albicans and a standard inoculum of streptomycin resistant E. coli cells suspended in 1 ml. of 0.85% saline. The animals of group 3 and 4 received the same graded inocula of C. albicans but no E. coli. At 2 and 5 days postinfection,stool samples were taken as described in procedure A. Each stool sample was emulsified in 1 ml. of 0.85% saline and 0.1 ml. of the resulting suspension was spread on a plate of Chapmans modification of Sabourauds maltose agar. After 48 hrs. incubation at 37 C,colonies of C. albicans were identified by their characteristic morphology. The 50% infective dose(ID50)was then determined according to the method of Reed and Muench(1938).

In all experiments additional stool cultures were made to determine the composition of the bacterial flora. This was done by streaking one loopful of the emulsified stool sample(same as used for Candida identification) on a plate of desoxycholate agar. After 24 hrs. incubation at 37 C,the gram negative bacterial flora was identified.

CHAPTER III

EXPERIMENTAL

Susceptibility of Normal Mice To C. albicans Infection. Thirty mice were divided into five groups of six mice each and inoculated by stomach tube with 0.1 ml. of increasing concentrations of cells of C. albicans suspended in 0.85% saline. No antibiotics were given in this experiment. Stool samples were taken from the mice, as described in procedure A, at various intervals up to forty days and cultured on Chapmans modification of Sabourauds maltose agar to detect C. albicans.

The results of this experiment are given in table 1. As can be seen, animals receiving inocula of 4.17×10^6 organisms or a smaller number did not excrete C. albicans at 48 hours after infection, while 5 out of 6 mice in the group receiving 1.67×10^7 cells excreted C. albicans and presumably were infected. Four of the five infected animals in this group remained positive for C. albicans for the duration of the experiment. This was interpreted as indicating that once an animal became infected it usually continued to harbor this microorganism. On the basis of this experiment, it was decided that stool samples taken at 2 and 5 days postinfection would be sufficient to determine whether or not an animal had developed an infection with C. albicans.

Effect Of Antibiotics On The Susceptibility Of Large And Small Mice

To C. albicans. It was of interest to know if age and size of the animal would be a factor involved in the susceptibility of mice to infection with C. albicans and also what organisms were eliminated from the intestinal flora by the antibiotic. In this experiment eighty mice were divided into eight groups of ten mice each. Four groups of each size received aureomycin and four groups served as antibiotic free controls. The procedure used in treating the animals was the same as that described in procedure A(except that groups 2 and 3, treated with streptomycin or aureomycin plus streptomycin respectively, were omitted).

The results of this experiment(Tables 2,2a,3,and 3a)indicated that size is not a significant factor in the susceptibility of mice to C. albicans. From the results of this experiment,it was decided to use large(25 to 30 gram) mice in the following experiments. In addition it was found that aureomycin was highly effective in reducing the number of E. coli,slightly effective against Aerobacter,and ineffective in reducing the incidence of Proteus in the treated animals(cf. tables 2a and 3a).

Choice Of Procedure For Determining The Effect Of Antibiotics On The Susceptibility Of Mice To C. albicans. It was of interest to know if the increased susceptibility demonstrated above could also be shown by quantitative determinations of the number of C. albicans recoverable from the stool of animals which had been given a standard infective dose(procedure B).

In the first experiment of this type,male Abrams mice weighing 25 to 30 grams were employed. Stool cultures were made as described in procedure A,at various intervals up to 16 days postinfection.

The results (Table 4) indicate that there was no significant difference in the excretion of C. albicans by antibiotic-treated and control mice up to 13 days after infection. The lower average numbers of C. albicans recovered in the control group were mainly due to those mice which were not infected at all or which showed a consistently low level of excretion. The standard error of the average counts (Table 4a) was for this reason extremely high and the T-Test (Goulden, 1939; Mainland, 1952) indicated that the differences between control and infected groups were statistically not significant. The infective dose of C. albicans given in this expe iment was relatively high (2.5×10^{8} cells). Another similar experiment was therefore performed with a lower infective dose (Table 5).

As can be seen from the data in table 5, the difference between control and antibiotic-treated groups was due to the large number of non-infected controls. Table 6 presents the results of a similar experiment of this type in which Rockland strain RAP mice were infected with an inoculum of 1.77×10^{6} cells. The number of C. albicans excreted by the controls and the group receiving aureomycin plus streptomycin were again not significantly different.

In summary, results obtained with procedure B (Tables 4 to 6) indicate that the number of C. albicans recoverable from the stools is not a good indicator of changes in the susceptibility of mice to oral infection with C. albicans during antibiotic therapy. This would suggest that the strain of Candida used was able to multiply almost equally well in the normal as in the antibiotic-treated mice. The only prerequisite for continued multiplication appeared to be an infective dose which was sufficiently large to enable the microorganism to establish itself in the intestine. All preliminary studies using procedures A or C(determination of the 50% infective dose)had consistently shown a higher susceptibility for antibiotic-treated mice — a finding which was confirmed by all subsequent experiments. One may consequently conclude that antibiotic treatment appeared to facilitate mainly the initial establishment of Candida in the mouse intestine,but that,after successful establishment,growth of the organism was comparatively less increased by the antibiotic. All further experiments of the present study were therefore carried out by determining the 50% infective dose of C. albicans(procedures A or C).

Effect Of Different Antibiotics On The Susceptibility Of Mice To C. albicans As Reflected By The 50% Infective Dose. The procedure followed in treating the animals was that described above for determining the 50% infective dose after administration of aureomycin,streptomycin,or aureomycin plus streptomycin(procedure A).

Individual results of three experiments are given in tables 7 to 11. Essentially the same results were obtained in each experiment indicating that mice treated with aureomycin,streptomycin,or with a mixture of aureomycin plus streptomycin were more susceptible to C. albicans than normal mice. It can be seen in table 11 that the increase in susceptibility caused by aureomycin is very similar to that obtained with streptomycin. Table 10 demonstrates that animals treated with aureomycin harbored a gram negative flora consisting of either Proteus,Aerobacter,or a combination of both. No gram negative bacteria could be recovered from animals treated with streptomycin. From these results it seems likely that there was no antagonism between Proteus, or Aerobacter, and C. albicans.

Effect Of E. coli On The Susceptibility Of Antibiotic Treated Mice To C. albicans. Because of the similarity in susceptibility of mice treated with either aureomycin or streptomycin, it was decided to investigate the effect of E. coli on the susceptibility of streptomycin treated mice to C. albicans. Streptomycin rather than aureomycin was used in these experiments because bacteria are readily made resistant to high concentrations of this antibiotic while this is very difficult to achieve with aureomycin. Unpublished data from this laboratory indicated that out of 25 strains of E. coli only two could be made resistant to 500 mcg./ml. aureomycin and that the resistant mutants had lost some of their original morphological and physiological characteristics.

Three experiments were performed using procedure C described previously. The results of these experiments are given in tables 12 to 15. A summary is presented in table 16. As can be seen, Experiments 1 and 2 indicate that the presence of the E. coli strains had no effect on the susceptibility of mice to C. albicans. Only Experiment 3 shows a higher susceptibility to Candida for mice which had not received E.coli. However, all three experiments indicate that the presence of E. coli did not reduce the susceptibility of antibiotic-treated mice to the low level shown by the antibiotic free controls. One must therefore conclude that antagonism between C. albicans and the strains of E. coli used was very slight if any.

Effect Of Aerobacter On The Susceptibility Of Antibiotic-Treated Mice To C. albicans. In one experiment originally designed to test for antagonism between E. coli and C. albicans, it was found that Aerobacter had completely overgrown the intestinal flora in all of the streptomycin treated animals. Presumably, these mice already harbored the resistant strain of Aerobacter in their intestines before this experiment was begun. This experiment was then used as a test for antagonism or synergism between Aerobacter and C. albicans.

The results of this experiment are given in table 17. The findings indicate that there was neither an antagonistic nor a synergistic effect between Aerobacter and C. albicans.

CHAPTER IV

DISCUSSION

As mentioned earlier, various theories have been advanced to explain the basic mechanism of superinfections with C. albicans during antibiotic therapy. As yet none of these suggested theories has been proven conclusively.

The studies reported here indicate that mice react in a similar fashion as human beings with respect to the increase in susceptibility to enteric infection with C. albicans during antibiotic therapy. As to how closely this process in mice parallels secondary infection with C. albicans during antibiotic therapy in man is not known. It was noticed in the present experiments that the animals did not show any of the clinical symptoms(fever, diarrhea, etc.)which may often, though by no means always, appear in secondary enteric Candida infections of human beings. However, the results obtained in the present studies suggest that these animals may be used as working models to study the primary mechanism by which C. albicans establishes itself in the intestinal tract during antibiotic therapy. One may perhaps assume that, even in human superinfections, Candida must reach a certain level of growth within the intestine, before it can proceed to invade or damage the superficial tissues, and thus produce the above mentioned symptoms. The superinfections in mice described in this thesis would then be analogous to the first stages of superinfections in man. Obviously all comparisons of the present experiments with human superinfections must be based on this assumption and are limited by it.

As mentioned earlier the present results suggest that the most pronounced stimulating effect of antibiotic treatment was on the primary establishment of a Candida population in the mouse intestine,while the subsequent growth of Candida was comparatively less affected by the drugs. It is not known whether this may also be true for superinfections in human beings. However,clinical studies of complications following antibiotic therapy indicate that Candida superinfections will persist in some cases even after the antibiotic therapy is discontinued(Gausewitz et al.,1951;Schaberg et al.,1955; Levy and Cohen,1955),while the infection will subside or disappear slowly after withdrawal of the antibiotic in others(Willcox,1951;Tomaszewski,1951; Robinson,1954). In a few cases the infection will recur,although less severely (Woods et al.,1951;Manheim and Alexander,1954). This may be taken to suggest some similarity between the mouse infections reported here and secondary enteric infections with C. albicans in certain human patients,who had apparently been predisposed to Candida infection by antibiotic therapy,but who remained infected wven in the absence of the predisposing factor.

The experiments summarized in table 11 indicate that there is an increase in the susceptibility of mice to C. albicans during treatment with either aureomycin,streptomycin,or a combination of both. This increase in susceptibility was reflected by a decrease in the 50% infective dose of C. albicans. It was also found that the 50% infective dose was similar for both the aureomycin treated animals and the streptomycin treated animals. Determinations of the gram negative enteric flora during antibiotic treatment indicated that animals given aureomycin harbored a gram negative flora consisting of either Proteus,Aerobacter,or a combination of both,while no gram negative bacteria could be recovered from the animals treated with streptomycin. These observations suggest that there was no antagonism between Proteus, or Aerobacter,and C. albicans.

In the experiments devised to study the effect of two E. coli strains on C. albicans(Tables 12,13,and 14),no appreciable antagonism could be demonstrated between these microorganisms. As mentioned earlier the E. coli strains had been selected on the basis of their strong antagonism to Shigella flexneri in enteric infections of mice(Hentges,1957). The negative results obtained in the present studies may therefore be interpreted to suggest that, if there is any E. coli:Candida antagonism at all,the mechanism of this antagonism must be different from that between E. coli and Shigella.

In summary then,the experiments with aureomycin and streptomycin treatment suggest,that there was no appreciable in vivo interaction - either antagonistic or synergistic - between C. albicans and Proteus or Aerobacter strains of the normal mouse flora. The failure to demonstrate in the present experiments any antagonism between E. coli and C. albicans,may further suggest that such a mechanism is not a factor in the determination of resistance or susceptibility of mice to enteric Candida albicans infection. However,further experiments employing different E. Coli strains(especially strains derived from resistant mice)are necessary to allow a definite conclusion. Furthermore, there is an obvious possibility of bacterial antagonism on the part of normal inhabitants of the intestinal tract such as gram positive sporeformers, anaerobes,lactobacilli,etc.,which were not included in the present experiments. Relevant studies are planned for the near future.

Another theory which appears quite plausible to the author,states that the mechanism of superinfection by Candida to be the result of direct stimulation of the fungus by antibiotics(Moore,1951). This theory is based on quantitative in vitro experiments with aureomycin. Pappenfort and Schnall (1951)and Huppert and Cazin(1953)also reported quantitative in vitro experiments with aureomycin which likewise indicated direct stimulation of Candida. The work presented here may also support this theory. However,Huppert and Cazin(1955a)could demonstrate in vitro stimulation of Candida only with three (aureomycin,neomycin,bacitracin)of the many antibiotics which are associated with superinfections in vivo. One reason for this may be that as yet no satisfactory procedure has been devised to reproduce in vitro the conditions under which Candida grows in the human or mouse intestine. Another possible explanation for the discrepancies between in vivo and in vitro results may be that antibiotics appear to facilitate mainly the initial establishment of Candida in the mouse intestine,while the subsequent growth of the organism is relatively less stimulated by the drugs. It may thus be possible that in vitro studies of the influence of antibiotics on the lag phase of growth of Candida(rather than on the final amount of growth)may lead to a better explanation of the in vivo results. Carpenter(1955)reported in vitro quantitative studies with Candida, employing various therapeutic agents, in which stimulation of the early growth rate rather than the final growth was noted.

CHAPTER V

SUMMARY

Rockland Farms strain RAP albino mice were used in most experiments described in this study. Two methods were used in determining the susceptibility of the animals to *C. albicans*: (A) graded inocula of *C. albicans* were given orally and the 50% infective dose determined on the basis of positive or negative stool cultures, or (B) a standard oral inoculum of *C. albicans* was given and the number of *C. albicans* in the stools determined by quantitative methods.

Results obtained in these experiments suggest that the antibiotics studied facilitated mainly the primary establishment of *C. albicans* in the intestinal tract of mice, while the subsequent growth of this microorganism was only slightly stimulated.

Streptomycin was found to increase the susceptibility of mice to *Candida* infection to a similar degree as aureomycin. This was interpreted to indicate that *Aerobacter* and *Proteus* species, which are eliminated by streptomycin but not by aureomycin, have little or no effect on the growth of *Candida* in the mouse intestine.

Tests for possible antagonism between strains of *E. coli* and *C. albicans* were carried out by feeding graded doses of *C. albicans* plus a

standard dose of the E. coli strain under study, and determining the 50% infective dose of C. albicans. Little or no antagonism was found in these experiments.

The significance of these findings for studies of the mechanism underlying overgrowth of C. albicans during antibiotic therapy is discussed, and some experimental approaches to this problem are suggested.

FIGURE 1

DENSITY CURVE OF E. COLI "25"

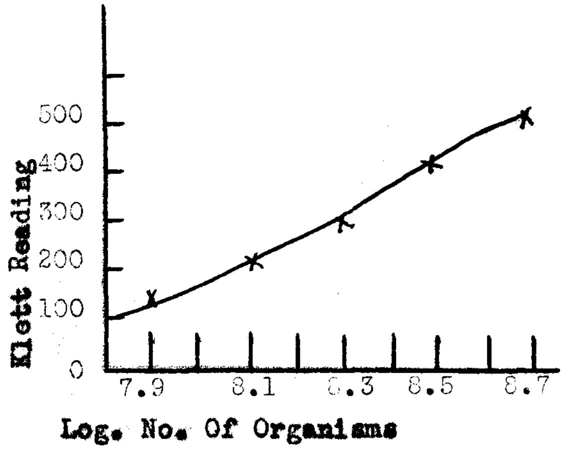

FIGURE 2

DENSITY CURVE OF E. COLI "5"

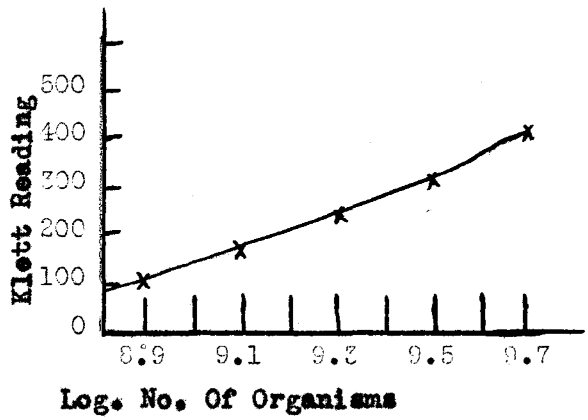

FIGURE 3

DENSITY CURVE OF <u>CANDIDA ALBICANS</u>

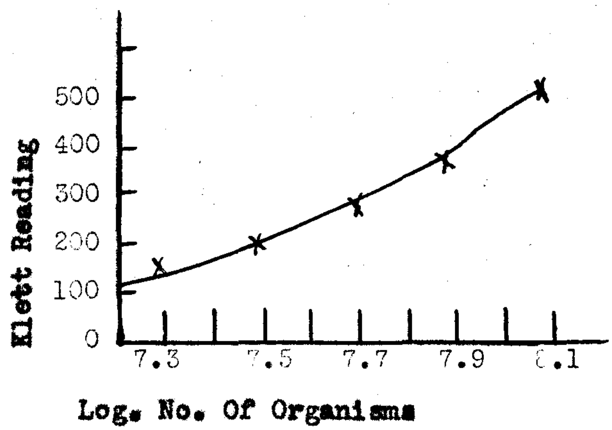

TABLE 1

EXCRETION OF <u>CANDIDA ALBICANS</u> BY MICE INOCULATED WITH INCREASING NUMBERS OF CELLS

Infective Dose of *C. albicans* *	No. of Mice In Group	Positive Stool Cultures On Days Postinfection 2	7	12	17	22	27	40
6.5 X 10^4	6	0/6+	0/6	0/6	0/6	0/6	0/6	0/6
2.6 X 10^5	6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
1.1 X 10^6	6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
4.2 X 10^6	6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
1.7 X 10^7	6	5/6	5/6	4/6	4/6	4/6	4/6	4/6

* Suspended in 1.0 ml. of 0.85% saline.

+ No. of cultures positive for *Candida* over total no. of cultures made.

TABLE 2

EFFECT OF ANTIBIOTICS ON THE SUSCEPTIBILITY TO ENTERIC CANDIDA INFECTION OF SMALL(12-14g.)MICE *

Group No.	Treatment	No. of Mice In Group	Infective Dose of *C. albicans*	Positive Stool Cultures On Days Postinfection 2	5	ID50 2 Days	5 Days
1a	Aureomycin	10	1.1×10^{3}	0/10 +	0/10		
1b	"	10	1.1×10^{5}	0/10	0/10		
1c	"	10	1.1×10^{6}	5/10	5/10		
1d	"	10	1.1×10^{7}	10/10	9/10	1.1×10^{6}	1.1×10^{6}
4a	Control	10	1.1×10^{3}	0/10	0/10		
4b	"	10	1.1×10^{5}	0/10	0/9		
4c	"	8	1.1×10^{6}	0/8	0/7		
4d	"	9	1.1×10^{7}	3/9	1/8	$>1.1 \times 10^{7}$	$>1.1 \times 10^{7}$

* Procedure A described under Methods was used in treating the animals.
(Groups 2 and 3 were omitted in this experiment)

+ No. of cultures positive for *Candida* over total no. of cultures made.

TABLE 3

EFFECT OF ANTIBIOTICS ON THE SUSCEPTIBILITY TO ENTERIC CANDIDA INFECTION OF LARGE(25-30g.)MICE *

Group No.	Treatment	No. of Mice In Group	Infective Dose of *C. albicans*	Positive Stool Cultures On Days Postinfection 2	5	ID50 2 Days	5 Days
1a	Aureomycin	10	1.4×10^3	0/10 +	0/10		
1b	"	10	1.4×10^5	1/10	1/10		
1c	"	10	1.4×10^6	8/10	5/9		
1d	"	10	1.4×10^7	8/9	8/9	1.0×10^6	1.4×10^6
4a	Control	9	1.4×10^3	0/9	0/8		
4b	"	9	1.4×10^5	0/9	0/9		
4c	"	8	1.4×10^6	0/8	0/8		
4d	"	9	1.4×10^7	5/9	5/8	1.4×10^7	1.2×10^7

* Procedure A described under Methods was used in treating the animals.
(Groups 2 and 3 were omitted in this experiment)

+ No. of cultures positive for *Candida* over total no. of cultures made.

TABLE 2A

EFFECT OF ANTIBIOTIC THERAPY ON THE BACTERIAL FLORA OF MICE *
(Same Animals As Described In Table 2)

Group No.	No. of Mice In Group	Treatment	E. coli	Proteus	Aerobacter
1a	10	Aureomycin	2/10 +	7/10	5/10
1b	10	"	0/10	10/10	4/10
1c	10	"	2/10	10/10	7/10
1d	10	"	0/10	10/10	7/10

TABLE 3A

EFFECT OF ANTIBIOTIC THERAPY ON THE BACTERIAL FLORA OF MICE *
(Same Animals As Described In Table 3)

Group No.	No. of Mice In Group	Treatment	E. coli	Proteus	Aerobacter
1a	10	Aureomycin	1/10 +	10/10	4/10
1b	10	"	1/10	9/10	7/10
1c	10	"	2/10	9/10	8/10
1d	9	"	0/9	8/9	6/9

* Tested on Desoxycholate Agar.

+ See Table 1, page 31.

TABLE 4

EFFECT OF AUREOMYCIN ON THE SUSCEPTIBILITY TO ENTERIC CANDIDA INFECTION OF ABRAMS LARGE(25-30g.)MICE *
(Experiment 1)

Days Postinfection

	5 Days	7 Days	10 Days	13 Days	16 Days
Group 1 - Aureomycin Treatment					
	10,000 #	410,000	100,000	60,000	270,000
	100	90,000	700	20,000	80,000
	7,000	200	30,000	7,000	140,000
	90,000	190,000	120,000	10,000	20,000
	30,000	50,000	310,000	11,000	280
	140,000	10,000	70,000	-	20,000
	70,000	70,000	160,000	60,000	890,000
	12,000	80,000	360,000	4,000	10,000
Average No.	46,137	112,525	143,950	21,500	166,285
Group 4 - Antibiotic Free Controls					
	20,000	-	-	-	-
	-	200,000	60,000	100	-
	40,000	10,000	40,000	60,000	24,500
	2,000	-	220	4,000	70,000
	30,000	160,000	30,000	60,000	-
	1,800	40,000	1,000	400	-
	50,000	200	200,000	30	140,000
	20,000	10,000	-	6,800	30,000
	20,475	49,150	18,902	16,416	33,062

* Procedure B was followed in treating the animals. - Infective Dose 2.5×10^8
(Groups 2 and 3 were omitted in this experiment)

No. of *Candida* recovered.

TABLE 4A

EFFECT OF AUREOMYCIN ON THE SUSCEPTIBILITY TO ENTERIC CANDIDA INFECTION OF ABRAMS LARGE(25-30g.)MICE
Statistical Analysis Of Results Obtained In Experiment 1 (Table 4)

Group 1 - Aureomycin Treatment	Days Postinfection 5	7	10	13	16	Summary Of 5 - 16
No. of Animals..................	8	8	8	8	8	40
Ave. No. of Candida............	46,137	112,525	143,950	21,500	166,285	100,375
Standard Deviation.............	-	134,713	128,362	-	-	162,800
Standard Error.................	-	48,111	44,262	-	-	25,841
t value........................	-	1.1	2.4	-	-	2.6

Group 4 - Antibiotic Free Controls

	5	7	10	13	16	5 - 16
No. of Animals..................	8	8	8	8	8	40
Ave. No. of Candida............	20,475	49,150	18,902	16,416	33,062	27,601
Standard Deviation.............	18,466	81,768	67,431	33,256	-	50,909
Standard Error.................	6,595	29,202	22,477	11,877	-	7,922

Std. Dev $= \sqrt{\frac{(X - M)^2}{N}}$ S.E. Diff. $= \sqrt{\frac{(S_1D_1)^2 + (S_2D_2)^2}{N - 1}}$ $t = \frac{\text{Ave. No.}_1 - \text{Ave. No.}_2}{\text{S.E. Diff.}}$

TABLE 5

EFFECT OF AUREOMYCIN AND STREPTOMYCIN ON THE SUSCEPTIBILITY TO ENTERIC CANDIDA INFECTION OF ABRAMS LARGE(25-30g.)MICE *
Experiment 2

	2 Days Postinfection	5 Days Postinfection
Group 1 - Aureomycin		
	90,000 #	20,000
	10,000	0
	8,000	0
	300	100
	30,000	1,000
	20,000	1,000
	170	30,000
	1,000	10,000
Ave. No.	17,718	18,011
Group 2 - Streptomycin		
	0	0
	1,300	600
	10	0
	100	160
	100	0
	0	0
	3,000	10
	0	40
	2,000	0
Ave. No.	723	90
Group 3 - Streptomycin plus Aureomycin		
	300	10
	2,000	1,000
	2,000	40,000
	70	1,000
	10,000	10,000
	1,000	300
	1,000	0
	0	0
	100	1,000
	10	40
Ave. No.	1,648	5,335
Group 4 - Antibiotic Free Controls		
	100	0
	0	0
	0	0
	100	0
	0	0
	0	0
	0	0
	0	0
	0	0
	0	0
Ave. No.	20	0

* Procedure B was used in treating the animals. # See Table 4, page 35.
(Infective Dose 3.4×10^6)

TABLE 6

EFFECT OF AUREOMYCIN AND STREPTOMYCIN ON THE SUSCEPTIBILITY TO ENTERIC <u>CANDIDA</u> INFECTION OF ROCKLAND (25-30g.)MICE *

	Group 3 - Aureomycin plus Streptomycin 2 Days Postinfection
	100 #
	20,000
	2,000
	30,000
	5,000
	400
	20,000
	30,000
	300
	10
Ave. No.	10,781

	Group 4 - Antibiotic Free Controls
	10
	100
	10,000
	0
	300
	2,000
	30,000
	300
	30,000
	10,000
Ave. No.	8,271

* Procedure B was followed in treating the animals. (Groups 1 and 2 were omitted in this experiment) Infective Dose 1.8 X 10^6

No. of <u>Candida</u> recovered.

TABLE 7

DETERMINATION OF THE 50% INFECTIVE DOSE FOLLOWING ANTIBIOTIC THERAPY *
(Experiment 1)

Group No.	No. of Mice In Group	Treatment	Infective Dose of C. albicans	Positive Stool Cultures On Days Postinfection 2	5	ID50 2 Days	5 Days
1a	5	Aureomycin	5.2×10^4	0/5 +	0/5		
1b	5	"	1.1×10^5	1/5	1/5		
1c	5	"	4.2×10^5	0/5	0/5		
1d	5	"	8.5×10^5	2/5	1/5		
1e	5	"	1.7×10^6	3/5	3/5	1.1×10^6	1.4×10^6
3a	5	Aureomycin plus Streptomycin	5.2×10^4	0/5	0/5		
3b	5	"	1.1×10^5	2/5	1/5		
3c	5	"	4.2×10^5	2/5	2/5		
3d	5	"	8.5×10^5	4/5	1/5		
3e	5	"	1.7×10^6	3/5	2/5	5.4×10^5	1.3×10^6
4a	4	Control	1.1×10^5	0/4	0/4		
4b	4	"	4.2×10^5	0/4	0/4		
4c	4	"	8.5×10^5	0/4	0/4		
4d	4	"	1.7×10^6	1/4	1/4	4.4×10^6	4.4×10^6
4e	4	"	3.3×10^6	0/4	0/4		

* Procedure A was followed in treating the animals (Group 2 was omitted).

+ No. of cultures positive for *Candida* over total no. of cultures made.

TABLE 8

DETERMINATION OF THE 50% INFECTIVE DOSE FOLLOWING ANTIBIOTIC THERAPY *
(Experiment 2)

Group No.	No. of Mice In Group	Treatment	Infective Dose of <u>C. albicans</u>	Positive Stool Cultures On Days Postinfection 2	5	ID50 2 Days	5 Days
1a	6	Aureomycin	8.0×10^4	2/6 +	0/6		
1b	6	"	3.3×10^5	2/6	0/6		
1c	6	"	1.3×10^6	2/6	0/6		
1d	6	"	4.6×10^6	6/6	5/6		
1e	6	"	1.8×10^7	4/6	4/6	1.3×10^6	3.9×10^6
2a	6	Streptomycin	8.0×10^4	0/6	0/6		
2b	6	"	3.3×10^5	2/6	1/6		
2c	5	"	1.3×10^6	2/5	2/5		
2d	6	"	4.6×10^6	5/6	4/6		
2e	6	"	1.8×10^7	5/6	5/6	1.6×10^6	2.7×10^6
3a	6	Aureomycin plus Streptomycin	8.0×10^4	1/6	1/6		
3b	6	"	3.3×10^5	1/6	0/6		
3c	6	"	1.3×10^6	3/6	3/6		
3d	6	"	4.6×10^6	5/6	5/6		
3e	6	"	1.8×10^7	6/6	6/6	1.04×10^6	1.3×10^6
4a	6	Control	8.0×10^4	0/6	0/6		
4b	6	"	3.3×10^5	0/6	0/6		
4c	5	"	1.3×10^6	0/5	0/5		
4d	6	"	4.6×10^6	1/6	1/6	1.1×10^7	1.1×10^7
4e	6	"	1.8×10^7	4/6	4/6		

* Procedure A was followed in treating the animals. + See Table 1, page 31.

TABLE 9

DETERMINATION OF THE 50% INFECTIVE DOSE FOLLOWING ANTIBIOTIC THERAPY *
(Experiment 3)

Group No.	No. of Mice In Group	Treatment	Infective Dose of <u>C. albicans</u>	Positive Stool Cultures On Days Postinfection 2	5	ID50 2 Days	5 Days
1a	6	Aureomycin	7.1×10^4	0/6 +	0/6		
1b	6	"	2.8×10^5	1/6	1/6		
1c	6	"	1.1×10^6	4/6	2/6		
1d	6	"	4.5×10^6	6/6	4/6		
1e	6	"	1.8×10^7	5/6	4/6	8.3×10^5	3.2×10^6
2a	6	Streptomycin	7.1×10^4	1/6	1/6		
2b	6	"	2.8×10^5	1/6	1/6		
2c	6	"	1.1×10^6	2/6	2/6		
2d	6	"	4.5×10^6	4/6	3/4		
2e	6	"	1.8×10^7	6/6	6/6	2.1×10^6	1.8×10^6
3a	6	Aureomycin plus Streptomycin	7.1×10^4	0/6	0/6		
3b	6	"	2.8×10^5	3/6	2/6		
3c	6	"	1.1×10^6	6/6	4/6		
3d	6	"	4.5×10^6	6/6	6/6		
3e	6	"	1.8×10^7	6/6	5/6	2.8×10^5	9.1×10^5
4a	6	Control	7.1×10^4	0/6	0/6		
4b	6	"	2.8×10^5	0/6	0/6		
4c	6	"	1.1×10^6	2/6	2/6	8.1×10^6	1.8×10^7
4d	6	"	4.5×10^6	2/6	2/6		
4e	6	"	1.8×10^7	3/6	1/6		

* Procedure A was followed in treating the animals.
\+ No. of cultures positive for <u>Candida</u> over total no. of cultures made.

TABLE 10

EFFECT OF ANTIBIOTIC THERAPY ON THE BACTERIAL FLORA OF MICE *
(Same animals as described in Table 6,Exp.3)

Group No.	No. of Mice In Group	Treatment	E. coli	Proteus	Aerobacter
1a	6	Aureomycin	1/6 +	5/6	0/6
1b	6	"	2/6	4/6	2/6
1c	6	"	0/6	6/6	0/6
1d	6	"	2/6	4/6	2/6
1e	6	"	2/6	5/6	3/6
2a	6	Streptomycin	0/6	0/6	0/6
2b	6	"	0/6	5/6	3/6
2c	6	"	2/6	0/6	0/6
2d	6	"	0/6	0/6	0/6
2e	6	"	1/6	1/6	0/6
3a	6	Aureomycin plus Streptomycin	0/6	0/6	0/6
3b	6	"	0/6	0/6	0/6
3c	6	"	0/6	0/6	0/6
3d	6	"	0/6	0/6	2/6
3e	6	"	0/6	0/6	0/6
4a	6	Normal	5/6	3/6	4/6
4b	6	"	6/6	5/6	6/6
4c	6	"	6/6	4/6	4/6
4d	6	"	6/6	2/6	5/6
4e	6	"	6/6	5/6	5/6

* Tested on desoxycholate agar. + No. of cultures positive over total no. of cultures made.

TABLE 11

50% INFECTIVE DOSE OF MICE FOLLOWING ANTIBIOTIC THERAPY. SUMMARY OF TABLES 7 TO 9.

No. of Mice	Treated	ID50 Days Postinfection 2	ID50 Days Postinfection 5	Factor Days Postinfection 2	Factor Days Postinfection 5
Experiment 1 (Table 7):					
25	Aureomycin	1.1×10^6	1.4×10^6	4.0 *	3.1
25	Aureomycin plus Streptomycin	5.4×10^5	1.3×10^6	8.2	3.4
20	Control	4.4×10^6	4.4×10^6	1	1
Experiment 2 (Table 8):					
30	Aureomycin	1.3×10^6	3.9×10^6	8.5	2.8
29	Streptomycin	1.6×10^6	2.7×10^6	6.9	4.1
30	Aureomycin plus Streptomycin	1.04×10^6	1.3×10^6	10.8	8.5
29	Control	1.1×10^7	1.1×10^7	1	1
Experiment 3 (Table 9):					
30	Aureomycin	8.3×10^5	3.2×10^6	9.8	5.6
30	Streptomycin	2.1×10^6	1.8×10^6	3.9	9.9
30	Aureomycin plus Streptomycin	2.8×10^5	9.1×10^5	28.6	19.7
30	Control	8.1×10^6	1.8×10^7	1	1

* Obtained by dividing ID50 of Control Group by ID50 of Treated Group.

TABLE 12

TEST FOR ANTAGONISM BETWEEN E. COLI AND CANDIDA ALBICANS * #
(Experiment 1)

Group No.	No. of Mice In Group	Treatment	No. of E. coli	E. coli Strain	Infective Dose of C. albicans	Positive Stool Cultures On Days Postinfection 2	ID50 2 Days
1a	6	Streptomycin	1.1×10^7	5	7.9×10^4	0/6 +	
1b	6	"	"	"	3.2×10^5	0/6	
1c	5	"	"	"	1.3×10^6	1/5	
1d	6	"	"	"	5.1×10^6	5/6	
1e	6	"	"	"	2.04×10^7	5/6	3.6×10^6
2a	6	Streptomycin	1.4×10^7	25	7.9×10^4	0/6	
2b	6	"	"	"	3.2×10^5	0/6	
2c	6	"	"	"	1.3×10^6	2/6	
2d	6	"	"	"	5.1×10^6	5/6	
2e	6	"	"	"	2.04×10^7	6/6	2.6×10^6
4a	6	Control	-	-	7.9×10^4	0/6	
4b	6	"	-	-	3.2×10^5	0/6	
4c	6	"	-	-	1.3×10^6	3/6	
4d	6	"	-	-	5.1×10^6	1/6	
4e	6	"	-	-	2.04×10^7	3/6	1.2×10^7

* Procedure C was followed in treating the animals(Group 3 was omitted in this exp.)

See Table 15,page 47 for determination of bacterial flora.

\+ No. of cultures positive for Candida over total no. of cultures made.

TABLE 13

TEST FOR ANTAGONISM BETWEEN <u>E. COLI</u> AND <u>CANDIDA</u> * #
(Experiment 2)

Group No.	No. of Mice In Group	Treatment	No. of *E. coli*	*E. coli* Strain	Infective Dose of *C. albicans*	Positive Stool Cultures On Days Postinfection 2	Positive Stool Cultures On Days Postinfection 5	ID50 2 Days	ID50 5 Days
1a	6	Streptomycin	9.1×10^6	5	8.9×10^4	1/6 +	1/6		
1b	6	"	"	"	3.4×10^5	2/6	1/6		
1c	6	"	"	"	1.4×10^6	2/6	2/6		
1d	6	"	"	"	5.4×10^6	5/6	3/6		
1e	6	"	"	"	2.2×10^7	6/6	5/6	1.4×10^6	3.6×10^6
2a	6	Streptomycin	7.8×10^6	25	8.9×10^4	0/6	0/6		
2b	6	"	"	"	3.4×10^5	2/6	0/6		
2c	6	"	"	"	1.4×10^6	4/6	4/6		
2d	6	"	"	"	5.4×10^6	4/6	4/6		
2e	6	"	"	"	3.2×10^7	6/6	6/6	1.1×10^6	1.4×10^6
3a	6	Streptomycin	-	-	8.3×10^4	0/6	0/6		
3b	6	"	-	-	3.3×10^5	3/6	1/6		
3c	6	"	-	-	1.3×10^6	2/6	2/6		
3d	6	"	-	-	5.4×10^6	5/6	4/6		
3e	6	"	-	-	2.2×10^7	5/6	4/6	1.6×10^6	3.8×10^6
4a	6	Control	-	-	8.3×10^4	0/6	0/6		
4b	6	"	-	-	3.3×10^5	1/6	1/6		
4c	6	"	-	-	1.3×10^6	1/6	1/6		
4d	6	"	-	-	5.4×10^6	4/6	3/6		
4e	5	"	-	-	2.2×10^7	3/5	2/5	5.1×10^6	3.3×10^7

* Procedure C was used in treating the animals.

See Table 15, page 47 for determination of bacterial flora.

\+ No. of cultures positive for <u>Candida</u> over total no. of cultures made.

TABLE 14

TEST FOR ANTAGONISM BETWEEN E. COLI AND CANDIDA * #
(Experiment 3)

Group No.	No. of Mice In Group	Treatment	No. of E. coli	E. coli Strain	Infective Dose of C. albicans	Positive Stool Cultures On Days Postinfection 2	5	ID50 2 Days	ID50 5 Days
1a	6	Streptomycin	1.1×10^7	5	3.9×10^4	0/6 +	0/6		
1b	6	"	"	"	1.6×10^5	0/6	0/6		
1c	6	"	"	"	6.4×10^5	2/6	1/6		
1d	6	"	"	"	2.6×10^6	5/6	2/6		
1e	6	"	"	"	1.1×10^7	6/6	2/6		
1f	6	"	"	"	4.1×10^7	6/6	5/6	1.3×10^6	1.0×10^7
2a	6	Streptomycin	1.2×10^7	25	3.9×10^4	0/6	0/6		
2b	6	"	"	"	1.6×10^5	0/6	0/6		
2c	6	"	"	"	6.4×10^5	1/6	1/6		
2d	6	"	"	"	2.6×10^6	3/6	1/6		
2e	6	"	"	"	1.1×10^7	5/6	3/6		
2f	6	"	"	"	4.1×10^7	5/6	5/6	4.4×10^6	8.7×10^6
3a	6	Streptomycin	-	-	5.8×10^4	0/6	0/6		
3b	6	"	-	-	2.3×10^5	6/6	4/6		
3c	6	"	-	-	9.3×10^5	5/6	3/6		
3d	6	"	-	-	2.7×10^6	5/6	4/6		
3e	6	"	-	-	1.1×10^7	5/6	4/6		
3f	6	"	-	-	4.4×10^7	4/6	4/6	2.2×10^5	1.5×10^6
4a	6	Control	-	-	5.8×10^4	0/6	0/6		
4b	6	"	-	-	2.3×10^5	0/6	0/6		
4c	6	"	-	-	9.3×10^5	1/6	0/6	1.1×10^7	2.3×10^7
4d	6	"	-	-	2.7×10^6	1/6	1/6		
4e	6	"	-	-	1.1×10^7	3/6	2/6		
4f	6	"	-	-	4.4×10^7	4/6	4/6		

* See Table 13, page 45. # See table 15, page 47. + See Table 1, page 31.

TABLE 15

GRAM NEGATIVE ENTERIC FLORA(TESTED ON DESOXYCHOLATE AGAR)OF MICE DESCRIBED IN EXPERIMENTS 1 - 3, TABLES 12 - 14

Experiment 1 (Table 12):

Group No.	Treatment	Flora On Days Postinfection 2	Flora On Days Postinfection 5
1	Streptomycin plus *E. coli* "5"	Only *E. coli*	Only *E. coli*
2	Streptomycin plus *E. coli* "25"	Only *E. coli*	Only *E. coli*
4	Normal Controls	Normal Flora	Normal Flora

Experiment 2 (Table 13):

Group No.	Treatment	Flora On Days Postinfection 2	Flora On Days Postinfection 5
1	Streptomycin plus *E. coli* "5"	Only *E. coli*	Only *E. coli*
2	Streptomycin plus *E. coli* "25"	Only *E. coli*	Only *E. coli*
3	Streptomycin	Sterile	Sterile
4	Normal Controls	Normal Flora	Normal Flora

Experiment 3 (Table 14):

Group No.	Treatment	Flora On Days Postinfection 2	Flora On Days Postinfection 5
1	Streptomycin plus *E. coli* "5"	Only *E. coli*	Only *E. coli*
2	Streptomycin plus *E. coli* "25"	Only *E. coli*	Only *E. coli*
3	Streptomycin	Sterile	Sterile
4	Normal Controls	Normal Flora	Normal Flora

TABLE 16

TEST FOR ANTAGONISM BETWEEN E. COLI AND CANDIDA
Summary of Tables 12 - 14

No. of Mice	Treatment	*E. coli* Strain	ID50(2 Days Postinfection)	ID50(5 Days Postinfection)	Factor *	
Experiment 1 (Table 12):						
29	Streptomycin	"5"	3.6×10^6	-	3.5	-
30	"	"25"	2.6×10^6	-	4.7	-
30	Normal Controls (No Antibiotic)	None	1.2×10^7	-	1	-
Experiment 2 (Table 13):						
30	Streptomycin	"5"	1.4×10^6	3.6×10^6	3.7	9.0
30	"	"25"	1.1×10^6	1.4×10^6	4.5	4.1
30	"	None	1.6×10^6	3.8×10^6	3.2	8.5
29	Normal Controls (No Antibiotic)	None	5.1×10^6	3.3×10^7	1	1
Experiment 3 (Table 14):						
36	Streptomycin	"5"	1.3×10^6	1.0×10^7	8.5	2.2
36	"	"25"	4.4×10^6	8.7×10^6	2.5	2.6
36	"	None	2.2×10^5	1.5×10^6	49.7	15.5
36	Normal Controls (No Antibiotic)	None	1.1×10^7	2.3×10^7	1	1

* Obtained by dividing ID50 of Control Group by ID50 of Treated Group.

TABLE 17

TEST FOR ANTAGONISM OF AEROBACTER AND CANDIDA *

Group No.	No. of Mice In Group	Treatment	Strain	Infective Dose of *C. albicans*	Positive Stool Cultures On Days Postinfection 2	ID50 2 Days	Factor # 2 Days
1a	6	Streptomycin	*Aerobacter*	6.9×10^4	1/6 +		
1b	5	"	"	2.8×10^5	0/5		
1c	6	"	"	1.1×10^6	1/6		
1d	6	"	"	4.3×10^6	3/6		
1e	6	"	"	1.7×10^7	6/6	3.3×10^6	3.3
2a	6	Streptomycin	*Aerobacter*	6.9×10^4	0/6		
2b	6	"	"	2.8×10^5	3/6		
2c	6	"	"	1.1×10^6	3/6		
2d	6	"	"	4.3×10^6	5/6		
2e	6	"	"	1.7×10^7	6/6	1.1×10^6	10.1
3a	6	Streptomycin	*Aerobacter*	6.5×10^4	0/6		
3b	6	"	"	2.6×10^5	1/6		
3c	6	"	"	1.04×10^6	3/6		
3d	6	"	"	4.2×10^6	5/6		
3e	6	"	"	1.7×10^7	6/6	1.0×10^6	10.3
4a	6	Controls (No Streptomycin)	-	6.5×10^4	0/6		
4b	6	"	-	2.6×10^5	0/6		
4c	6	"	-	1.04×10^6	1/6		
4d	6	"	-	4.2×10^6	0/6		
4e	6	"	-	1.7×10^7	5/6	1.1×10^7	1

* Procedure C was used in treating the animals.

Obtained by dividing ID50 of Control Group by ID50 of Treated Group.

+ No. of cultures positive for *Candida* over total no. of cultures made.

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APPROVAL SHEET

The thesis submitted by James William Messer has been read and approved by three members of the Department of Microbiology.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

January 7, 1958
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