6-1964

Relation between Use of Lincomycin and Outgrowth of Yeast

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RELATION BETWEEN USE OF LINCOMYCIN AND OUTGROWTH OF YEAST

by

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A thesis presented to the
Faculty of the School of Graduate
Studies in partial fulfillment
of the
Degree of Master of Arts

Western Michigan University
Kalamazoo, Michigan
June, 1964

ACKNOWLEDGMENTS

The author wishes to express his gratitude to The Upjohn Company for the use of laboratory space and equipment during this experimental study. He also gives thanks to Drs. J.E. Grady, L.E. Rhuland and O.K. Sebek for advice, criticism and assistance in the course of this work.

Special recognition is given to Dr. J.J. Josten of Johns Hopkins University for his guidance and encouragement, especially during the experimental phase of this work.

The investigator gratefully acknowledges the advice and interest of Mr. Merrill R. Wiseman, Dr. Jean O. Lawrence and Dr. Richard Brewer of the Department of Biology at Western Michigan University in the preparation of this paper.

Sincere appreciation is given to Mrs. E.A. Minster for her kind and patient interest shown during the preparation of this manuscript.

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INTRODUCTION

The use of antibiotics has increased the average life expectancy of mankind. They have prevented and cured infectious diseases that previously terminated in death. Consequently, recovery is often prompt and complete.

Though a benefit to mankind, the use of antibiotics in the treatment of infectious disease has also been accompanied by new problems. For example, treatment of a primary infection such as a bacterial pneumonitis with a broad spectrum antibiotic, may result in the death of some of the bacteria. More than one species of bacteria, however, are often found in the infected area. If such be the situation, a reduction of the antibiotic sensitive bacteria may result in establishment of a resistant bacterial population. Pathogens present in this resistant population may then cause a secondary infection. This infection is often more serious than that caused by the primary pathogen (1). The antibacterial effects of the drug are also exerted in non-infected tissues of the body. If given orally, some of the drug is usually absorbed through the small intestine, circulated by the blood stream and excreted. Quantities of the drug, however, may not be absorbed, but discharged through the large intestine. While it remains in the large intestine, some of the antibiotic may continue to exert its antibacterial action. Normally a balanced microflora is present in the lower

intestine of man. If the drug inhibits sensitive organisms in the large intestine, the creation of an ecologic vacuum is possible. This vacuum may be filled by other microorganisms.

The organisms which are most likely to fill this vacuum are antibiotic-resistant bacteria and/or other non-sensitive microorganisms. Fungi imperfecti are pathogens which under such conditions often grow-out resulting in a secondary infection. Secondary superimposed fungus infections caused by yeast cells, especially Candida albicans, can be sequelae of antibiotic therapy (2,3). Other yeast species which are normally present as saprophytes in man can give rise to serious infections (4). Important conditions which may be involved in such yeast infections are abnormally high numbers of these organisms and the general physiological condition of the host.

Candida sp. is not specifically confined to man or even the animal body. Extensive evidence given in the recent review of the genus Candida by Skinner and Flecher (5) shows that these organisms are found in several non-animal habitats. Examples of some of these habitats are soil, insects, mushrooms, dung, shrimp, fruit and exudates of trees. With such a distribution, contact with this organism is frequent. In addition, the exposure of the sensitive intestinal microflora to an antibiotic would result in an even greater chance of yeast outgrowth because of the elimination of the sensitive organisms. The incidence of Candida albicans as part of a normal human flora may then become of medical importance and should not be minimized. This organism has been

isolated from the oral cavity of 6 to 24 per cent normal humans in several surveys (6,7,8). Schnoor (9) was able to isolate Candida species from 33 per cent of 314 fecal cultures. About half of the isolated species were Candida albicans. These studies show that yeast organisms are present in a significant percentage of the normal human population.

The incidence of Candida sp. in human feces has been high following antibiotic therapy for bacterial infections. Due to this yeast occurrence, several studies were done whose purpose was to find an explanation for the yeast outgrowth. Over several years many hypotheses were published. Examples of the major theories are given below (10).

1. The antibiotic may stimulate the growth of fungi which are present in small numbers in the nasopharynx and gastrointestinal tract (11,12). Woods (2) and Hesseltine (13) oppose this theory.

2. The antibiotic may antagonize body defense mechanisms which operate to control pathogenic fungi. A report in the literature indicates that chlorotetracycline interferes with phagocytosis (14).

3. The antibiotic may disrupt the balance among microorganisms in the body. The number of yeasts present is generally of a lower order than the number of bacterial cells. Bacterial flora are presumed to contribute substances which inhibit yeast growth (15,16). If significant bacterial suppression occurs, the fungi

grow readily. This imbalance causes a disruption of the physiology of the body especially concerning availability and adsorption of essential nutrilites. For instance, it is known from animal experiments that feeding sulfonamides (17), or streptomycin (18), can result in deficiencies of the vitamin B group. This disruption is due to microbial suppression, but the mechanism is not known.

Although the above hypotheses may be plausible for any given incidence, the mechanisms whereby fungi acquire pathogenic properties after antibiotic administration is still obscure, and other unknown factors may be equally important (19).

PURPOSE

The observation was made during clinical trials with the antibiotic lincomycin that an outgrowth of yeast occurred in the large intestine of several subjects. This paper is concerned with the study of the direct effects of lincomycin on yeast growth, and particularly the effects of lincomycin on species of yeast which were isolated from human feces.

Lincomycin was discovered in fermentation broths of Streptomyces lincolnensis var. lincolnensis sp. n., and its biological activity has been reported by Mason, Dietz and DeBoer (20). Isolation and characterization of lincomycin were reported by Herr and Bergy (21). Lewis, Clapp and Grady (22) studied the in vitro and in vivo antibiotic activity.

Lincomycin can be degraded into two separate components by hydrolytic processes. These moieties are distinguished from one another by chromatography, and are referred to here as Compounds I and II (23).

Several clinical studies which tested the effect of lincomycin on the human intestinal microbial flora showed a high incidence of yeast outgrowth (24,25,26). From one of these studies, four representative yeasts were isolated using colony morphology as the identifying characteristic. These cultures were further studied taxonomically, and two

different genera were identified. They were the pathogenic Candida albicans and the non-pathogenic Saccharomyces sp. (27). It should be noted that the incidence of Saccharomyces sp. was greater than that of Candida albicans. The reverse is frequently found as a consequence of antibiotic therapy.

METHODS AND MATERIALS

Media

The media used in these experiments were either complex or synthetic. The compositions are given in Tables 1 and 10.

Assay Organism

Saccharomyces sp. and *Candida albicans* were used as the assay organisms. These organisms were isolated from a subject who took part in a clinical study previously referred to in the introduction.

Stock Culture and Assay Procedures

Stock suspensions were prepared by growing the test organism in 125 milliliter (ml) Erlenmeyer flasks containing 100 ml of brain-heart infusion broth (Difco), pH 7.4. The culture was incubated at 30°C for 20 to 24 hours without aeration and stored at 5°C for one week.

Immediately before an experiment 7 ml of the stock suspension was centrifuged at 1800 rpm for 20 minutes, washed with 10 ml of sterile distilled water and resuspended in 2 ml of test medium. Aliquots of 0.1 ml of the washed cell suspension were used which represented a standard inoculum size of 10^6 cells per ml. Departures from this routine are noted.

The culture tubes were side-arm colorimeter tubes designed by Cohen and Barner (28). The final volume per tube in each test was 10 ml.

Optical density measurements were made at 660 millimicrons (mμ) in a Beckman Model B Spectrophotometer.

Viable cell counts were made using Sabouraud's Dextrose (Difco) or Brain-Heart Infusion Agar (Difco). The spread plate technique was used, i.e., the cultures were diluted 10-fold in sterile distilled water and 0.1 ml aliquots were removed and spread on the surface of the medium by use of a "L" shaped glass rod.

Chromatography

The chromatograms were developed in two systems; butanol-acetic acid-water 12:3:5 and butanol-pyridine-water 6:4:2. After development in the appropriate system for 18 hours, the chromatograms were sprayed with either of two diagnostic sprays: 0.1 per cent potassium permanganate ($KMNO_4$) or 0.1 per cent ninhydrin. $KMNO_4$ allows detection of compound II and ninhydrin compound I.

Bioautography

Bioautography trays (54 x 20 centimeter plastic trays) were prepared by pouring into them 200 ml of test medium cooled to 45°C and seeded with 10^6 cells per ml of assay organism. Paper chromatograms were overlaid on the tray for 10 minutes; the trays were then incubated at 30°C for 24 to 48 hours.

Radioactivity Measurements

Tritiated lincomycin (lincomycin-H^3) was provided by Dr. J. J. Josten, formerly of the Upjohn Research Laboratories; this preparation was approximately 98 per cent pure as determined by chromatography and bioassay. Specific activity was 3.8 microcuries per milligram (μc per mg). Yeast cells were grown 16 hours in the presence of lincomycin-H^3, and recovered from the medium by centrifugation. These cells were then examined for lincomycin incorporation as described below. The supernatants were chromatographed for detection of lincomycin hydrolysis by radioactivity scanning and bioautography.

A Vanguard Model 880 Autoscanner was used for scanning the chromatograms.

The cells were washed in trichloroacetic acid (TCA) to remove cellular components not incorporated into cell structure, then assayed directly for radioactivity in a Packard Tri-Carb Liquid Scintillation Spectrometer System Model 314 AX. The scintillation solvent was a modification of the diotol solution of Herberg (29). The efficiency of counting tritium for this particular instrument was about 10 per cent. Standard scintillating techniques were used, i.e., 0.1 ml aliquots from the TCA washes and cell suspensions were suspended in 10 ml counting solvent and contained in 12 ml counting vials. The contents of the vials were then assayed for radioactivity.

RESULTS

Selection of Assay Organism and Test Medium

In view of the higher *Saccharomyces* sp. than *Candida albicans* population noted in clinical studies, a strain of *Saccharomyces* sp. was selected as the primary assay organism for use in this study.

Three media were investigated for ability to support yeast growth. These media consisted of Brain-Heart Infusion Broth (BHIB) (Difco) and Sabouraud's Liquid Medium (SLM) (Difco) and a synthetic glucose-salts (GS) medium, Table 1. The temperature of incubation was 30°C (30). The effects of aeration and pH on yeast growth were also investigated.

Effect of Aeration

The growth response of *Saccharomyces* sp. to various lincomycin concentrations was tested first in BHIB with aeration. Figure 1 shows that after 7 hours incubation, growth in the presence of lincomycin was approximately 2-fold greater than that of the control. Viable cell counts determined at 0 and 24 hours (Table 2) showed that this growth was the result of an increase in cell numbers rather than a change in optical density due to cellular elongation or swelling. This observation was considered preliminary evidence that lincomycin stimulated an increase in population of *Saccharomyces* sp.

Table 3 shows an analogous pattern of growth response with nonaerated cultures. Although the stimulation was not as great as that observed in the aerated culture, it was highly reproducible.

A growth response due to lincomycin was observed in synthetic medium. Figure 2 shows that this response occurred in aerated and in nonaerated cultures. The extent of growth at 20 hours, however, was greater in the nonaerated than the aerated cultures.

The Effect of pH

Tables 4 and 5 are compilations of the data from experiments in which the pH of the medium was varied. These data show that the test organism grew best in the pH range 5.0 - 5.5. When the pH was increased above 5.5, a decrease in control growth was noted. At pH 9.0, this decrease was greatest. The stimulation of yeast growth by lincomycin was best observed when the pH was 6.0 or greater. Figures 3 and 4 show that stimulation at pH 9.0 and 8.0 required concentrations of lincomycin greater than 100 micrograms (mcg) per ml.

When a selective yeast medium was used, SLM (Difco), presterilization pH 9.0, stimulation of yeast growth occurred at concentrations of 5 and 10 mg lincomycin per ml (Table 6). This stimulation was detectable at 9 hours, but at 24 hours stimulation was not observable (optical density).

It should be noted that the lincomycin concentrations used in these experiments to stimulate yeast growth were comparable to those

detected in fecal samples obtained from clinical studies (31).

The experiments described above, showed that the growth of Saccharomyces sp. was stimulated by appropriate concentrations of lincomycin. This stimulation was observed in aerated and nonaerated cultures. It was seen in both complex and synthetic media at pH values of 6.0 and greater.

Physiology of Lincomycin Stimulation

In the experiments described below, synthetic medium, pH 6.8 and nonaerated cultures were used. In certain of the experiments, vitamins were added to the basal synthetic medium. Unless otherwise noted, these vitamins were contained in the medium in the concentrations as shown in Table 10.

Two supplementary requirements which may function in the areas of metabolism are the need for organic ions and trace elements, and vitamins and "grow factors". The possible role of lincomycin in vitamin or growth factor areas was studied.

Table 7 shows the effect of lincomycin on yeast cells in the lag phase of the growth cycle. Lincomycin (10 mg per ml) was added at 0 hours, and viable cell counts were made at hourly intervals through 5 hours. In the tube which contained lincomycin, there was no decrease in the viable count. At 5 hours the viable cell count had increased by 0.11 log units which indicates the beginning of the exponential

phase. In the tube which contained no lincomycin, a decrease in viable cell count was noted during the 5-hour lag period. At 1 hour, this count had decreased 0.16 log units. An additional decrease of 0.30 log units was observed at 4 hours. At 5 hours the cells had increased 0.30 log units, and it was evident that the cells were then entering the exponential phase of growth.

Figure 5 shows the optical density measurements which were made for the experiment described above. Lincomycin (10 mg per ml) was added at 0 and 5 hours. It can be seen (Figure 5) that at 12-1/4 hours the greatest growth occurred in the tube which contained the lincomycin added at 0 hours. The addition of lincomycin at 5 hours also caused an increase in growth at 12-1/4 hours - approximately 0.75 optical density units greater than the control growth. This test demonstrated that lincomycin stimulated yeast growth during the exponential phase and during the lag phase of the growth cycle.

In another test, synthetic medium was inoculated with 10^3 to 10^7 yeast cells per ml. The cells were incubated for 336 hours, and viable cell counts were taken at 0 and at each 24-hour period. Serial transfers were made from the previous 24-hour growth into fresh medium using 1 to 10 dilutions. The results of this experiment showing restoration of normal cell growth by lincomycin can be seen in Table 8.

The initial 4-day period showed an increase in cell numbers. This increase was followed by a stable period, and then decreases in

cell population. On day 5, 10 mg lincomycin per ml was added to each tube. The tubes were incubated for 84 hours when a viable cell count was made. At the end of this period, the viable cell count had increased in all tubes - the increase varied from 4 log units to less than 1. On day 9 and 10, the test organism was transferred into fresh medium containing 10 mg lincomycin per ml. At the end of this period, an increase in viable cells was noted in each tube. Therefore, the increase observed at 84 hours was due to the presence of lincomycin in the medium and not a result of extended incubation. During the next 4-day period, days 11, 12, 13 and 14, the concentration of lincomycin in the test medium was decreased daily by 10-fold. Within this period, the increases in the viable cell counts were proportional to the lincomycin concentration. In fact, at a concentration of 1 mcg lincomycin per ml cell death occurred.

These results suggest that lincomycin prevents cell death for periods up to 8 days under conditions which did result in death. Depletion of essential metabolites and trace elements can be considered as causes of this yeast cell death. Other explanations, such as cellular alterations in the internal pH concentration may allow increased protein utilization for cell reproduction. It is not possible at this time, therefore, to state the exact mechanism of this response.

Lincomycin Lots

Five different lots of lincomycin were tested for stimulatory

activity on yeast cells (Table 9). All lots were tested at a concentration of 10 mg lincomycin per ml. Figure 6 shows that all lots stimulated yeast growth regardless of chemical form, i.e., needles or rhomboid crystals. The "stimulatory factor," therefore was not dependent on a particular form or lot of the antibiotic.

Utilization of Lincomycin as a Substrate

It was of interest to determine whether the yeast growth stimulation resulted from utilization of lincomycin as a carbon source. This possibility was tested in several experiments, and Figure 7 is representative of these tests.

It can be seen in Figure 7 that the test organism in the presence of 1 or 20 mg lincomycin per ml and 3 per cent glucose grew to a 4-fold greater extent by 22 hours than the control. When the medium contained concentrations of 20, 10, 5, 2.5 and 1 mg lincomycin per ml but no glucose, the yeast failed to grow. These results indicate that lincomycin was not utilized as a substrate, and did not substitute for glucose as a carbon source.

Incorporation of Lincomycin and Metabolism

The prevention of cell death by lincomycin (Table 8) made it logical to determine whether the antibiotic was metabolized to an essential substrate and/or incorporated by the cells.

In the experiment represented in Figure 3, all tubes containing lincomycin were centrifuged to remove cells and supernatants were

assayed for lincomycin. The assay technique used was the method of Hanka et. al. (32). One hundred per cent of the lincomycin activity was recovered in the supernatants and thus suggested that lincomycin was not metabolized or incorporated by the cells.

Supernatants from the tubes containing 10 mg lincomycin per ml (Figure 2) were chromatographed; controls were lincomycin, compound I and compound II dissolved in the test medium. The latter two compounds are the two components into which lincomycin can be hydrolyzed. All compounds were applied at 100 mcg per spot. The results indicated that lincomycin had not been cleaved.

Bioautographs of duplicate chromatograms were made on agar trays which were inoculated with Saccharomyces sp. and Sarcina lutea. The chromatogram from the culture supernatant gave a spot of Sarcina lutea inhibition which corresponded to one given by known lincomycin. There was no evidence of yeast stimulation.

At this time, a pure preparation of tritiated lincomycin became available. This preparation was assayed, and its specific radioactivity was found to be 3.8 μc per mg. This preparation was used subsequently in order to achieve greater sensitivity than was allowed by bioactivity measurements alone.

In the preliminary isotopic studies, the test organism was grown in the presence of 9.9 mg carrier lincomycin per ml and 100 mcg lincomycin-H^3 per ml.

Cells were removed by centrifugation after 16 hours incubation. The supernatants were chromatographed and scanned for radioactivity. Lincomycin had not been cleaved into compounds I or II as indicated by inability to demonstrate any radioactivity other than intact lincomycin. The cells were extracted with cold 10 per cent TCA, washed twice in TCA and then assayed for radioactivity. No radioactivity could be detected in the cells, thus lincomycin was not incorporated into the cell structure.

In a test of similar design in which the specific activity of the tritiated lincomycin addition was increased to 19 μc per ml, no detectable lincomycin metabolism or incorporation occurred.

Relationship of Lincomycin to Vitamins, Amino Acids and Nucleotides

Vitamins

At least six nutrilites are known to be essential for the growth of yeasts. They are thiamine, biotin, inositol, niacin or nicotinamide, pantothenic acid and pyridoxine. Para-aminobenzoic acid has also been shown to be essential for the growth of certain strains of Saccharomyces cerevisiae (33).

The effects of biotin, dethiobiotin, thiamine, lipoic acid and a multiple vitamin solution on yeast growth were examined. These compounds were tested alone and in combination with lincomycin.

Figure 8 shows the results of an experiment which examined lincomycin as a substitute for biotin and/or dethiobiotin. This experiment demonstrated that concentrations of 0.1 - 10 mcg biotin per ml and 0.1 - 10 mcg dethiobiotin per ml did not stimulate yeast growth to the same extent as lincomycin. There was no additive response with the lincomycin biotin or dethiobiotin supplements. Similarly, (Figure 9) no stimulation was obtained with lipoic acid. Thiamine, however, with lincomycin caused an additive stimulatory response.

The effect of a multiple vitamin solution (Table 10) on yeast growth was tested with the results shown in Figure 10. There was stimulation with the vitamin solution or lincomycin alone and the lincomycin-vitamin combination caused a 3-fold increase in growth. Thus, a clearly synergistic effect due to the lincomycin-vitamin combination occurred. The mechanism is not readily apparent.

Amino Acids

The possibility that lincomycin was involved in amino acid metabolism was tested with the results shown in Figure 11. The composition of the added amino acid mixture is given in Table 11. Growth in the presence of the amino acid mixture was less than that in the presence of lincomycin. When lincomycin and amino acids were combined, the growth response was not greatly enhanced. When the yeast cells were supplied with vitamins and amino acids, the growth response was greatly stimulated. Lincomycin with vitamins and amino acids resulted in the greatest growth response.

It will be noted that the control culture, i.e., no supplement, did not grow. This failure of control growth had been observed in several experiments of like design. The growth failure appears to be linked to a lack of available growth factors necessary for cell replication such as vitamins or trace elements.

Lincomycin appeared not to be involved in amino acid metabolism per se.

Nucleotides

The possibility that lincomycin was involved in nucleotide metabolism was studied as shown in Figure 12. Nucleosides were added to the medium (Table 12). These results showed that the nucleosides added did not greatly affect the growth response. When the nucleosides and lincomycin were added together, the growth response was the same as that with lincomycin alone. Lincomycin, therefore, did not appear to be involved in nucleotide metabolism.

Lincomycin and Compound I and II Studies

It was of interest to determine whether the stimulation was a property of lincomycin per se or could be attributed to either compound I or II, the two principal products of lincomycin hydrolysis.

Preliminary studies indicated that compound I stimulated yeast growth while compound II inhibited yeast growth. Compound II, at 10 mg per ml, changed the pH of the medium from 6.8 to 8.0, and

gradually precipitated over a 24-hour period. In subsequent experiments when the pH of the medium was corrected to 6.8 before inoculation, no inhibition, but rather stimulation was observed. Lincomycin and compound I are freely soluble and have no affect on the pH of the medium.

In a comparison of compound I and II and intact lincomycin, a test was performed with equal concentrations of the compounds. The results can be seen in Table 13. In the case of compound II the pH was corrected to 6.8 at 0 hours.

Lincomycin supplementation effected a dose response; the greatest stimulation occurred at 14.7 mg per ml, and correspondingly lesser stimulation at each lower concentration. At 29.5 mg per ml, the stimulation was less than at 14.7 mg per ml. This diminished response at the increased concentration may be due to compound I in the lincomycin molecule since as noted below, higher concentration of this compound showed inhibition of cell growth.

Compound I caused the greatest stimulation at the lower concentration used, and was only slightly stimulatory at 1.85 mg per ml. As the concentration of compound I was increased, inhibition of the test organism at each increase was observed.

Compound II elicited stimulatory responses at all concentrations tested.

Figure 13 is representative of studies which showed that neither compound I nor II was utilized as substrates. In the presence of 3 mg of each compound per ml, the yeast failed to grow. When 3 mg glucose per ml was added, the yeast grew normally.

These studies indicate that supplementation of the medium with lincomycin, compound I or compound II causes stimulation of yeast growth.

Candida albicans Stimulation by Lincomycin

Table 14 shows that Candida albicans growth was stimulated by lincomycin. Stimulation occurred in the presence of 10, 5, 2.5 and 1 mg lincomycin per ml. The greatest stimulation took place at a lincomycin concentration of 10 mg per ml, the least at 1 mg per ml.

DISCUSSION

The studies described in this paper were prompted by the results of clinical experiments in which outgrowths of yeast were observed in the feces of patients after lincomycin administration (34). It is a purpose of this study to examine the mechanism of yeast outgrowth as it pertained to lincomycin therapy in particular, and antibiotic therapy in general.

The data from this study showed that lincomycin stimulated the growth of yeast at concentrations greater than 100 mcg per ml; 10 mg per ml was optimal. Stimulation was observed in complex and synthetic media, and was pH dependent. The stimulation could be observed at pH values as low as 6.0, but when the pH was raised to 8.0 the better stimulation was observed (Figure 4). At alkaline pH values, the control growth decreased proportional to pH increase (Tables 4 and 5). The stimulation was observed under both aerated and nonaerated conditions, but was better seen when the cultures were not aerated (Figure 2).

The *in vitro* and *in vivo* conditions allow the following correlations: the concentrations of lincomycin in fecal samples are comparable to the concentrations which caused stimulation *in vitro* of yeast growth (31); the contents of the lower bowel contain sufficient nutrilites for microbial growth as evidenced by the presence of the large numbers of microorganisms in the feces; the pH of the digestive tract

is acid nearest the stomach, and becomes progressively more alkaline in the large intestine in which the microflora are located; the contents of the large intestine are also under reduced oxygen tension. Based on the *in vitro* data given in previous sections, similar *in vivo* conditions would allow yeast to multiply. The specific mechanisms of yeast stimulation are not, however, readily apparent from the *in vitro* results.

In a recent paper by Luckey (35), the stimulatory activity of antibiotics was discussed. Antibiotics have been shown to promote growth in some animals. It has been demonstrated, for instance, that antibiotics decrease the apparent vitamin requirements and increase the utilization of protein by animals (36). It is interesting to note that lincomycin may stimulate the growth of yeast by a mechanism which is related to vitamins. When both lincomycin and vitamins were present, a synergistic relationship clearly existed (Figure 10). It was suggested that lincomycin might function by preventing the leakage of vitamins necessary for normal cell growth (Tables 7 and 8). Thus, lincomycin under such conditions would protect any further decrease in the apparent vitamin requirements and allow for normal cell replication.

The possibility of a lincomycin-thiamine interrelationship exists (Figure 9). Lincomycin could act as a thiamin*ase* antagonist. For example, inhibition of thiaminase activity by lincomycin would allow utilization by yeast cells of exogenous thiamine. Although thiaminase

activity was not shown in this study, thiaminase has been demonstrated in yeasts (37). In these yeasts an equilibrium between synthesis and inactivation of thiamine was postulated. Lincomycin, therefore, may function by establishing a temporary imbalance in this system. A consequence would be stimulated yeast growth.

Steward (38) discusses several compounds which are involved in the control of plant cell growth. The chemical structures of these growth control factors are dissimilar, but some contain nitrogen atoms in their structure. The emperical formula of lincomycin has two nitrogen atoms as shown by Herr and Bergy (21). It is possible that these nitrogen atoms are of importance in the mechanism of yeast growth stimulation. Some other subtle structural feature may, however, account for the stimulation of this yeast growth.

To determine whether yeast stimulation was a unique property of lincomycin or a general characteristic of antibiotic compounds, several commonly used antibiotics were tested for stimulatory properties under conditions that resulted in stimulation with lincomycin.

Antibiotics are grouped into specific categories on the basis of their biochemical action on bacterial cells. These categories are as follows: 1) suppressors of cell wall synthesis, 2) suppressors of protein synthesis, 3) disrupters of lipoprotein membranes. In group (1) penicillin, cycloserine and bacitracin are placed; chloramphenicol, the tetracyclines, erythromycin, puromycin and streptomycin are placed

in group (2); group (3) contains gramicidin S, the polymyxins, subtilin, and tyrocidin (39).

Penicillin, bacitracin, chloramphenicol, tetracycline, erythromycin, streptomycin, polymyxin B and neomycin were examined. These tests showed that all of the above antibiotics except erythromycin and neomycin stimulate the growth of yeast. In addition, the polyamines spermine and cadaverine also stimulate yeast growth. A comparison of the structures of these compounds reveals no common entity.

The mechanism of lincomycin yeast stimulation may lie in areas of cell physiology that are not amenable to the direct approaches attempted in this study. Such areas would include cellular control and permeability.

In cellular control mechanisms the "glucose effect" is included. That is, in an inducible enzyme system, the induction of the enzyme is inhibited by glucose. When glucose is removed, enzyme synthesis occurs. This glucose effect has been long recognized in bacteria. It has recently been shown that the same effect occurs in yeast (40). If the glucose effect applies to the study described in this paper, lincomycin may reverse glucose repression and cause an apparent stimulation of growth.

It is possible that lincomycin has a role in permeability, i.e., making the membrane more permeable to nutrilites which are necessary for enhanced growth. This effect would be related to the particular

molecular configuration of lincomycin in contact with the cell membrane.

Finally, lincomycin may affect the intracellular environment of the cell in a non-specific manner, for instance, alteration of H-ion concentraction. This alteration would reverse the activity of a rate limiting reaction, and cause a stimulation of yeast growth.

SUMMARY

The influences of lincomycin on yeast growth were studied. The *in vitro* data are summarized below.

Lincomycin stimulates the growth of yeast at concentrations greater than 100 mcg per ml. This stimulation is consistent at a concentration of 10 mg per ml, which concentration is comparable to that found in clinical fecal samples. In synthetic and complex media, the stimulation occurred under aerated and nonaerated conditions, and was pH dependent.

Lincomycin does not function as a carbon source, nor was lincomycin metabolized or incorporated by yeast cells.

When the basal medium was supplemented with amino acids or nucleosides, yeast stimulation was not as great as with lincomycin alone. A synergistic relationship between lincomycin and vitamins was demonstrated.

Two lincomycin degradation products stimulated the growth of yeast.

Lincomycin prevented a decrease in viable cell count during the lag phase of growth.

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TABLE 1

Composition of Yeast Synthetic Basal Medium in Per Cent

Compound	Per Cent
$(NH_4)_2\ SO_4$	0.4
KH_2PO_4	0.2
$MgSO_4$	0.05
Glucose for Carbon Source added aseptically	3.0
Sterile distilled H_2O QS to 100 ml.	

FIGURE 1

The Effect of Various Lincomycin Concentrations on Saccharomyces sp. Growth. BHIB pH 7.4 30°C + Aeration

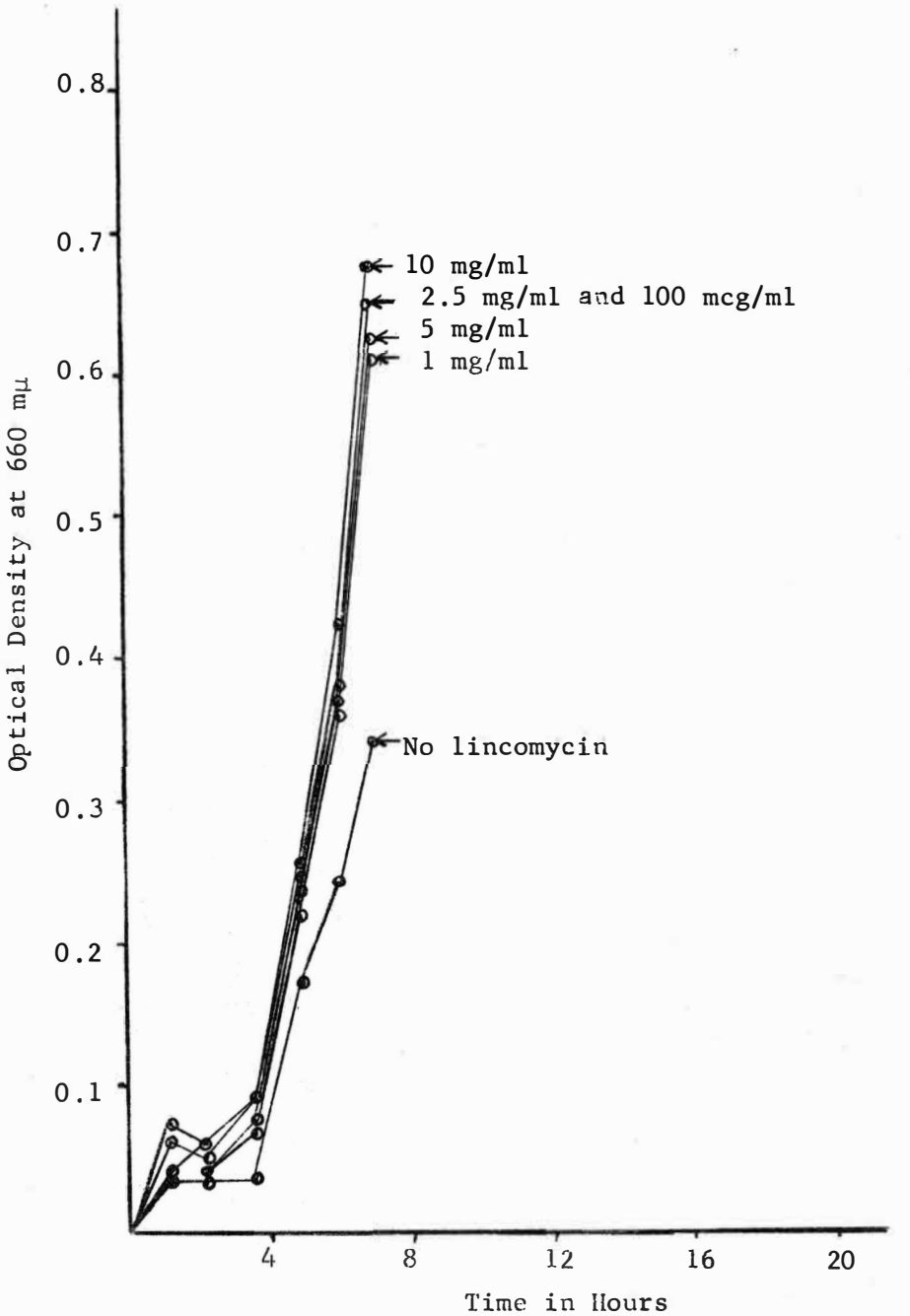

TABLE 2

The Effect of Lincomycin on the Extent of Yeast Growth
Viable Cell Counts/ml[1]

Conc. of Lincomycin/ml	0 Hour	24 Hour
Growth Control (No Lincomycin)	2.23×10^6	1.27×10^8
1 mg	2.23×10^6	1.88×10^8
2.5 mg	2.23×10^6	2.08×10^8
5 mg	2.23×10^6	1.59×10^8
10 mg	2.23×10^6	3.26×10^9

1 = Counts recorded after 48 hours incubation at 30° C. Cells were plated on brain-heart infusion pH 7.4.

TABLE 3

The Effect of Lincomycin on Nonaerated Yeast Growth
Optical Density at 660 mμ[1]

Conc. of Lincomycin/ml	0 Hour	6-1/2 Hour
10 mg	.000	.377
5 mg	.000	.387
100 mcg	.000	.387
50 mcg	.000	.377
5 mcg	.000	.357
Growth Control (No Lincomycin)	.000	.319

1 = Optical density measurements were made in a Beckman Model B Spectrophotometer. Cells were grown in brain-heart infusion broth pH 7.4.

FIGURE 2

The Effect of Various Lincomycin Concentrations on Yeast Growth.
Synthetic Medium pH 6.8 30°C

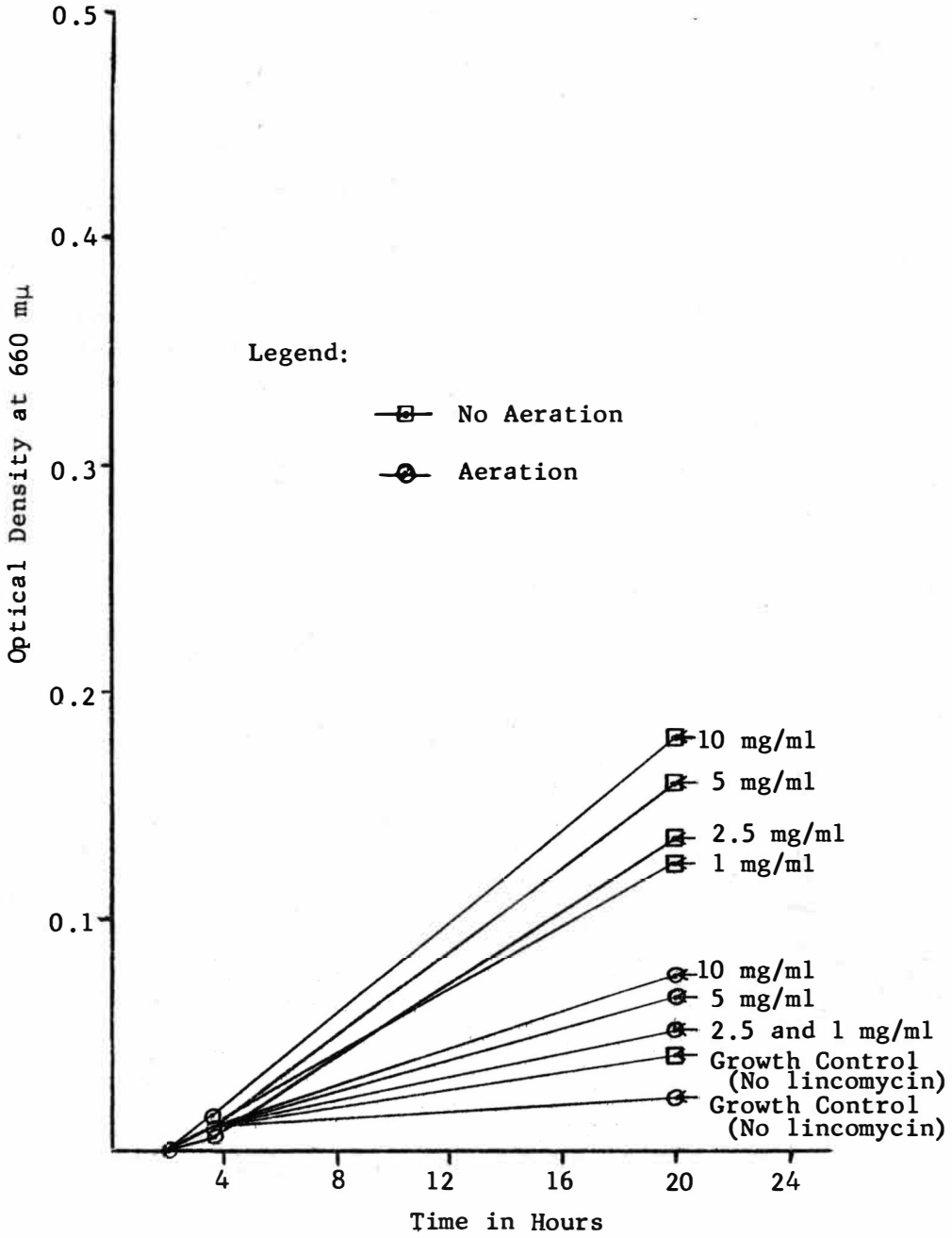

TABLE 4

The Effect of pH on Growth Response of Saccharomyces sp. to Lincomycin in Brain-Heart Infusion Broth[1]

Conc. of Lincomycin/ml	0 Hours	4 Hours	6 Hours	7 Hours	pH
10 mg	.000	.076	.357	.569	5.5
5 mg	.000	.086	.367	.585	5.5
100 mcg	.000	.086	.377	.585	5.5
No Lincomycin	.000	.061	.347	.585	5.5
10 mg	.000	.027	.155	.377	7.5
5 mg	.000	.027	.138	.357	7.5
100 mcg	.000	.032	.125	.275	7.5
No Lincomycin	.000	.018	.102	.259	7.5
10 mg	.000	.080	.191	.284	8.5
5 mg	.000	.029	.164	.243	8.5
100 mcg	.000	.048	.108	.138	8.5
No Lincomycin	.000	.048	.108	.144	8.5
10 mg	.000	.027	.071	.125	9.0
5 mg	.000	.018	.046	.081	9.0
100 mcg	.000	.013	.022	.027	9.0
No Lincomycin	.000	.022	.032	.036	9.0

1 = Optical density measurements were made in a Beckman Model B Spectrophotometer at 660 mμ. Cells were grown in brain-heart infusion broth with no aeration.

TABLE 5

The Effect of pH on Growth Response of Saccharomyces sp. to Lincomycin in Synthetic Medium[1]

Conc. of Lincomycin/ml	0 Hr.	4 Hr.	6 Hr.	24 Hr.	pH
10 mg	.000	.009	.018	.187	5.0
5 mg	.000	.011	.018	.187	5.0
2.5 mg	.000	.004	.022	.187	5.0
1 mg	.000	.009	.022	.187	5.0
No Lincomycin	.000	.009	.022	.119	5.0
10 mg	.000	.013	.022	.201	6.0
5 mg	.000	.004	.022	.161	6.0
2.5 mg	.000	.004	.018	.161	6.0
1 mg	.000	.009	.022	.113	6.0
No Lincomycin	.000	.009	.022	.125	6.0
10 mg	.000	.022	.056	.387	7.0
5 mg	.000	.022	.051	.377	7.0
2.5 mg	.000	.022	.046	.328	7.0
1 mg	.000	.013	.032	.276	7.0
No Lincomycin	.000	.018	.009	.018	7.0

1 = Optical density measurements were made in a Beckman Model B Spectrophotometer at 660 mμ. Cells were grown with no aeration.

FIGURE 3

The Growth Response of Saccharomyces sp. to Various Concentrations of Lincomycin. BHIB pH 9.0 30°C No Aeration

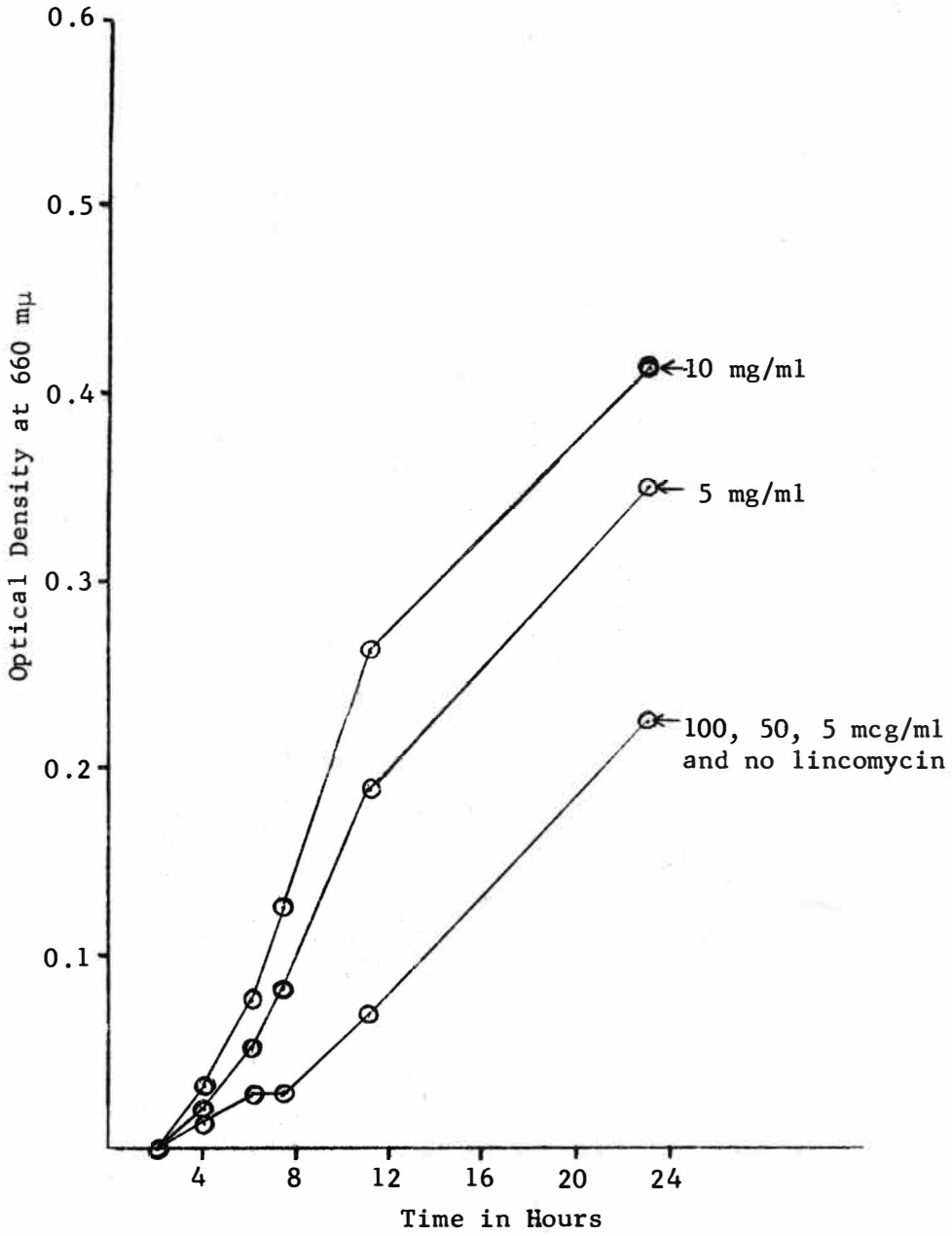

FIGURE 4

Dose Response of Saccharomyces sp. to Various Concentrations of Lincomycin Synthetic Medium pH 8.0 30°C No Aeration

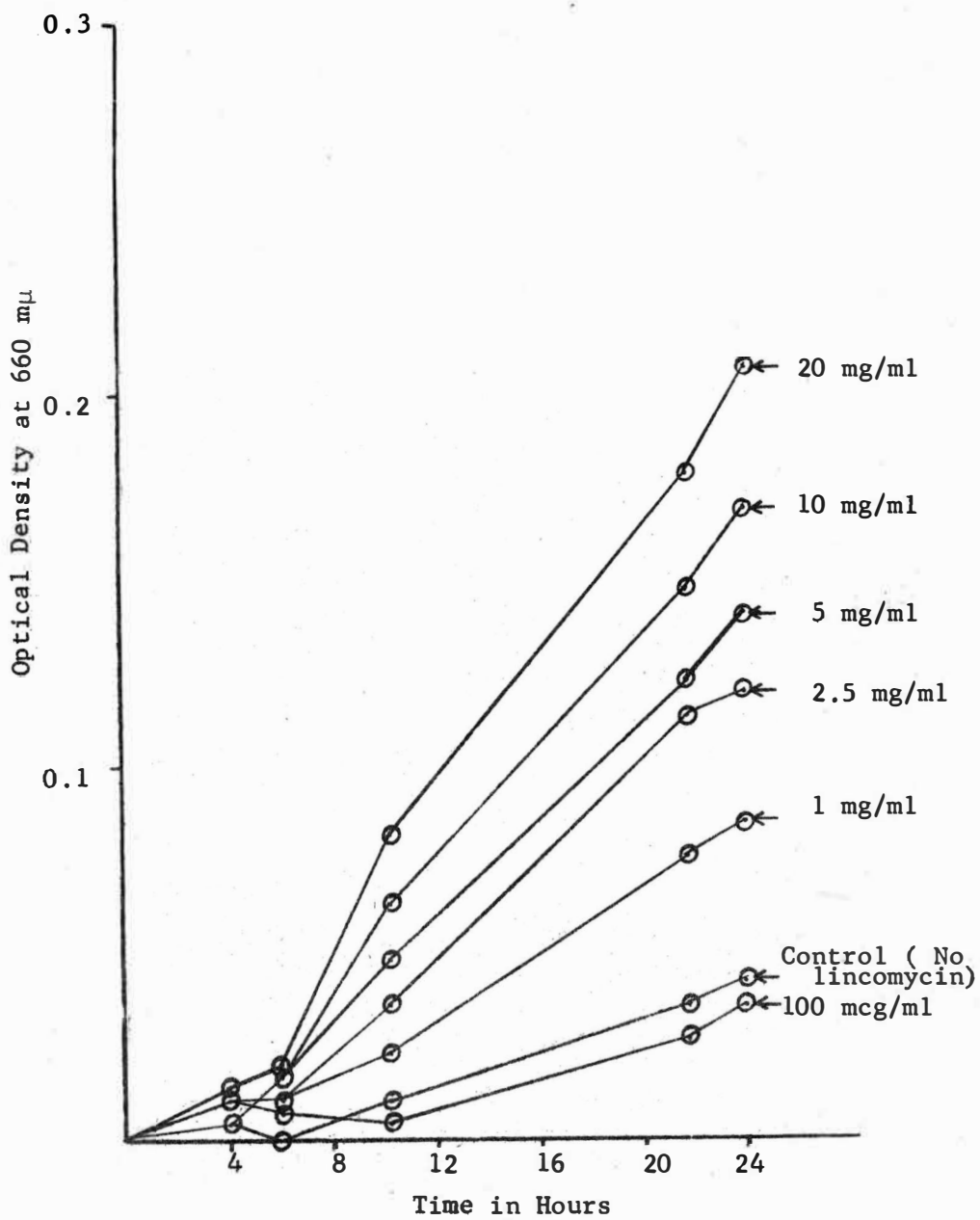

TABLE 6

Growth Response of Saccharomyces sp. to Various Concentrations of Lincomycin in Sabouraud's Liquid Medium pH 9.0[1]

Conc. of Lincomycin/ml	0 Hr.	3½ Hr.	5½ Hr.	9 Hr.	24½ Hr.
10 mg	.000	.031	.168	.959	1.50
5 mg	.000	.022	.137	.886	1.50
100 mcg	.000	.013	.097	.796	1.50
50 mcg	.000	.013	.097	.796	1.50
5 mcg	.000	.013	.102	.796	1.50
Growth Control (No Lincomycin)	.000	.013	.108	.796	1.50

1 = Optical density measurements were made in a Beckman Model B Spectrophotometer at 660 mμ. Cells were grown at 30°C. No aeration.

TABLE 7

The Effect of Lincomycin on Yeast Cells in the Lag Phase of Growth[1]

Time	Log of Viable Cells/ml	
	Lincomycin 10 mg/ml	No Lincomycin
0 Hours	5.60	5.66
1 Hour	5.61	5.50
2 Hour	5.59	5.50
3 Hour	5.68	5.50
4 Hour	5.66	5.20
5 Hour	5.71	5.50

1 = Counts recorded after 24 hours incubation at 30°C. Cells were plated on Sabouraud's Glucose Agar.

FIGURE 5

The Effect of 10 mg/ml Lincomycin Added at 0 and 4 Hours. Synthetic Medium + Vitamins pH 6.8 30°C No Aeration

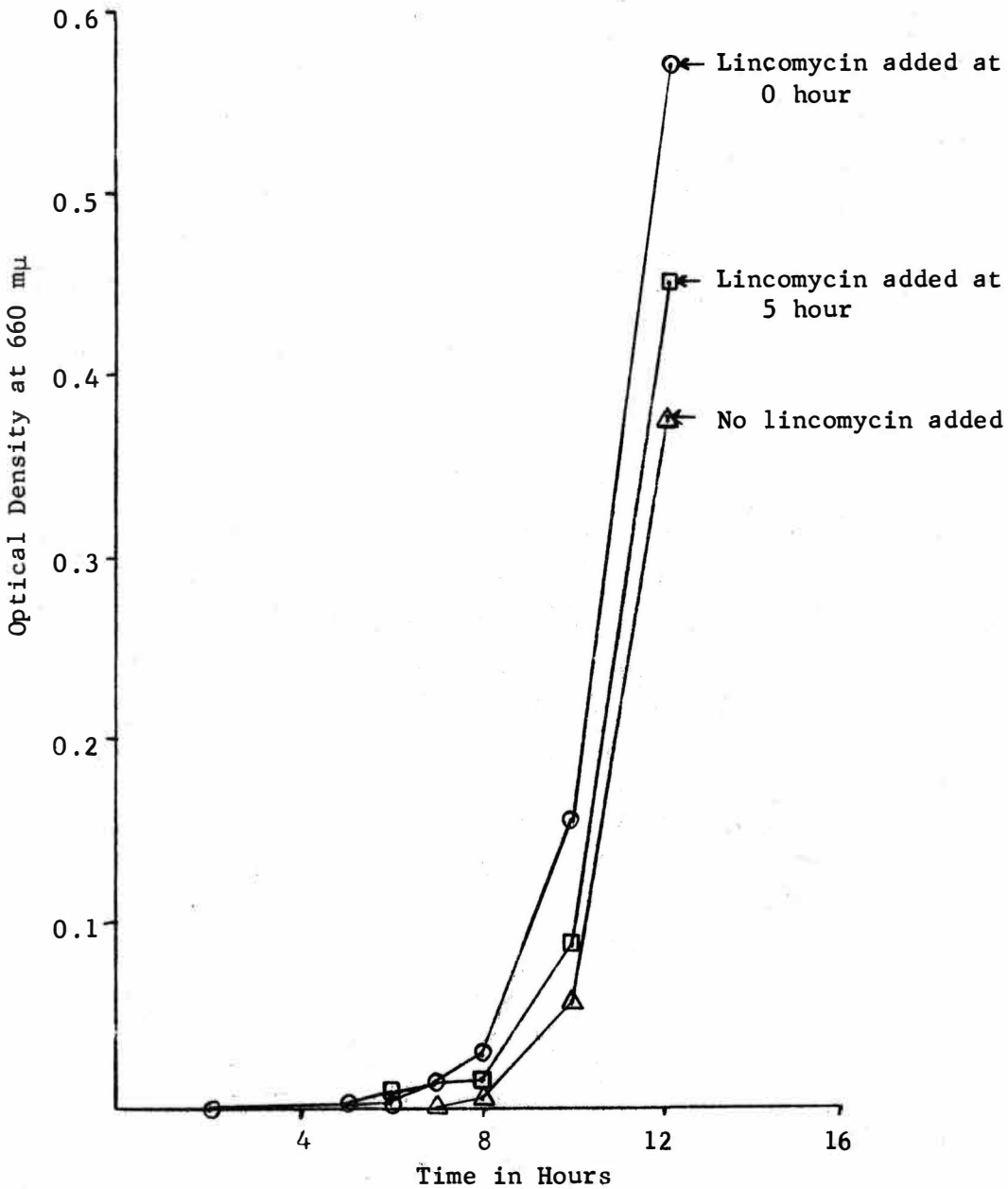

TABLE 8

Restoration of Normal Growth by Lincomycin. Results Listed as Log of Viable Cells/ml

Day No.		Tube Number						
		1	2	3	4	5	6	7
#1	0 Hr.	7.48	7.43	6.74	6.21	5.63	4.62	3.52
	24 Hr.	8.21	8.13	7.68	7.20	6.58	5.39	4.36
#2	0 Hr.	7.21	7.13	6.38	6.20	5.58	4.39	3.36
	24 Hr.	7.22	7.15	6.54	6.21	5.53	4.50	3.64
#3	0 Hr.	6.22	6.15	5.54	5.21	4.53	3.50	2.64
	24 Hr.	6.31	6.16	5.59	5.17	4.45	3.54	2.71
#4	0 Hr.	5.31	5.16	4.59	4.17	3.45	2.54	1.71
	24 Hr.	5.43	4.89	-	3.91	3.07	2.41	0.57
#5	0 Hr.	-	-	-	-	-	-	-
(10mg/ml)*	24 Hr.	-	-	-	-	-	-	-
#6	0 Hr.	-	-	-	-	-	-	-
(10mg/ml)*	24 Hr.	-	-	-	-	-	-	-
#7	0 Hr.	-	-	-	-	-	-	-
(10mg/ml)*	24 Hr.	-	-	-	-	-	-	-
#8	0 Hr.	-	-	-	-	-	-	-
(10mg/ml)*	24 Hr.	5.80	5.45	5.04	5.02	5.03	4.73	4.13
#9	0 Hr.	4.80	4.45	4.04	4.02	4.03	3.73	4.13
(10mg/ml)*	24 Hr.	5.01	4.83	4.68	4.72	4.60	4.47	4.18
#10	0 Hr.	4.01	3.83	3.68	3.72	3.60	3.47	3.18
(10mg/ml)*	24 Hr.	4.53	4.60	4.56	4.57	4.67	4.15	4.09
#11	0 Hr.	3.53	3.60	3.56	3.57	3.67	3.15	3.09
(1 mg/ml)*	24 Hr.	4.33	4.26	4.26	4.30	4.39	4.10	4.00
#12	0 Hr.	3.33	3.26	3.26	3.30	3.39	3.10	3.00
(100 mcg/ml)*	24 Hr.	3.76	3.79	3.49	3.74	3.91	3.67	3.55

TABLE 8 (Cont'd)

Day No.		Tube Number 1	2	3	4	5	6	7
#13	0 Hr.	2.76	2.79	2.49	2.74	2.91	2.67	2.55
(10mcg/ml)*	24 Hr.	3.10	2.89	2.53	3.11	2.95	2.64	2.71
#14	0 Hr.	2.10	1.89	1.53	2.11	1.95	1.64	1.71
(1mcg/ml)*	24 Hr.	0.84	0.95	0.47	0.47	0.60	0.47	0.69

* = Conc. of Lincomycin added.

TABLE 9

Information of Coded Preparations and Pre-Growth pH Values

Code	Information	Pre-Growth pH
6702-ADA-128.1	Concentrated Mother Liquor[1]	8.3
14,121-19	Lincomycin lot of questionable purity[2]	6.8
6177-MEB-86	Lincomycin Form II[3]	6.8
5449-DMW-66	Lincomycin Form I[4]	6.8
14,121-13	Lincomycin lot used in clinical studies	6.8
14,121-14	Lincomycin lot used in previous yeast studies	6.8
Growth Control		6.8

[1] Substance remaining in mother liquor after lincomycin crystallization.

[2] Failed specifications because a red color was detected in the preparation.

[3] Crystalline form = needles.

[4] Crystalline form = rhomboid.

FIGURE 6

The Growth Response of Saccharomyces sp. to 10 mg/ml Concentrations of Different Lincomycin Preparations. Synthetic Medium pH 6.8 + Vitamins 30°C No Aeration

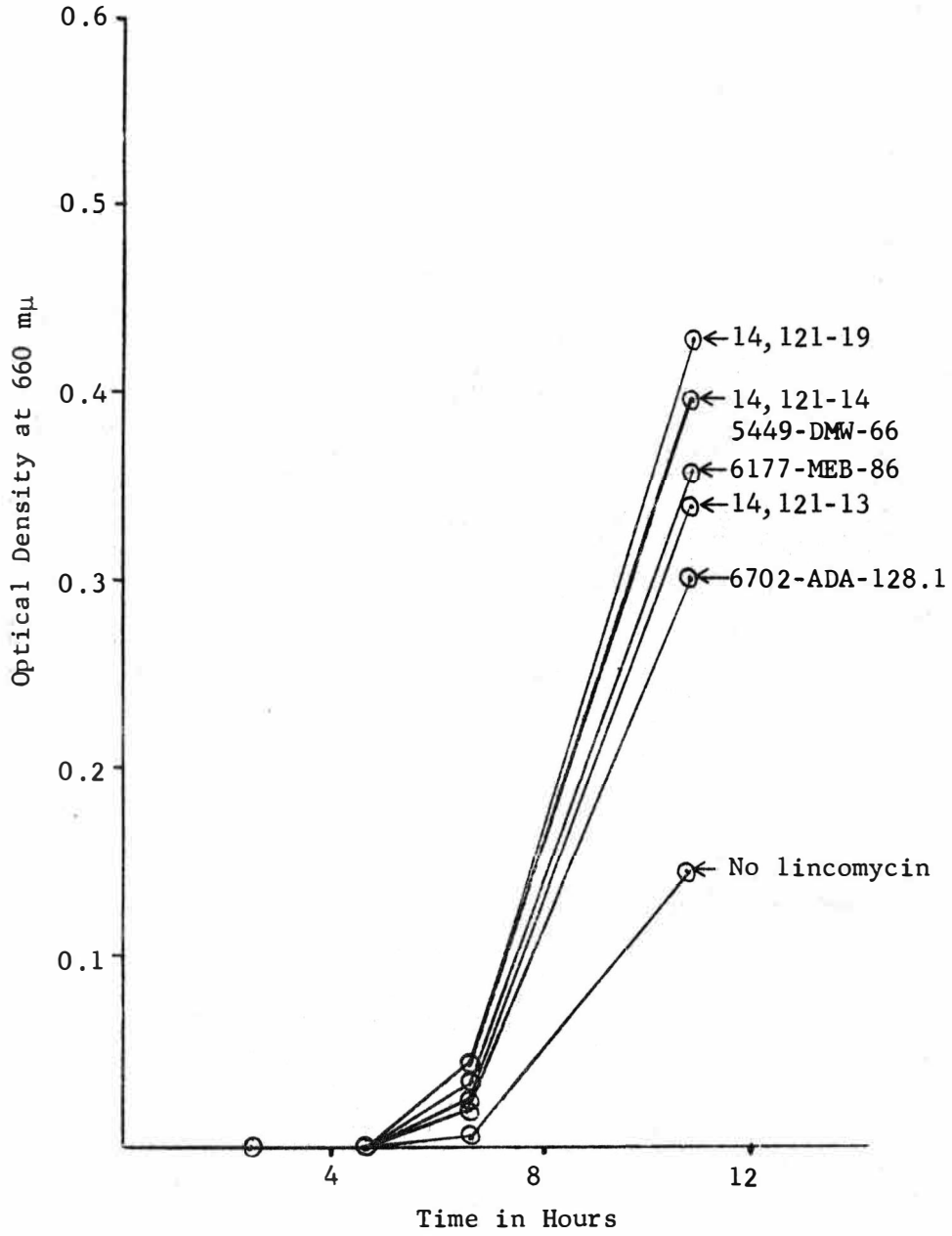

FIGURE 7

Lincomycin Utilization as a Substrate Test Organism Saccharomyces sp.
Synthetic Medium pH 6.8 30°C No Aeration

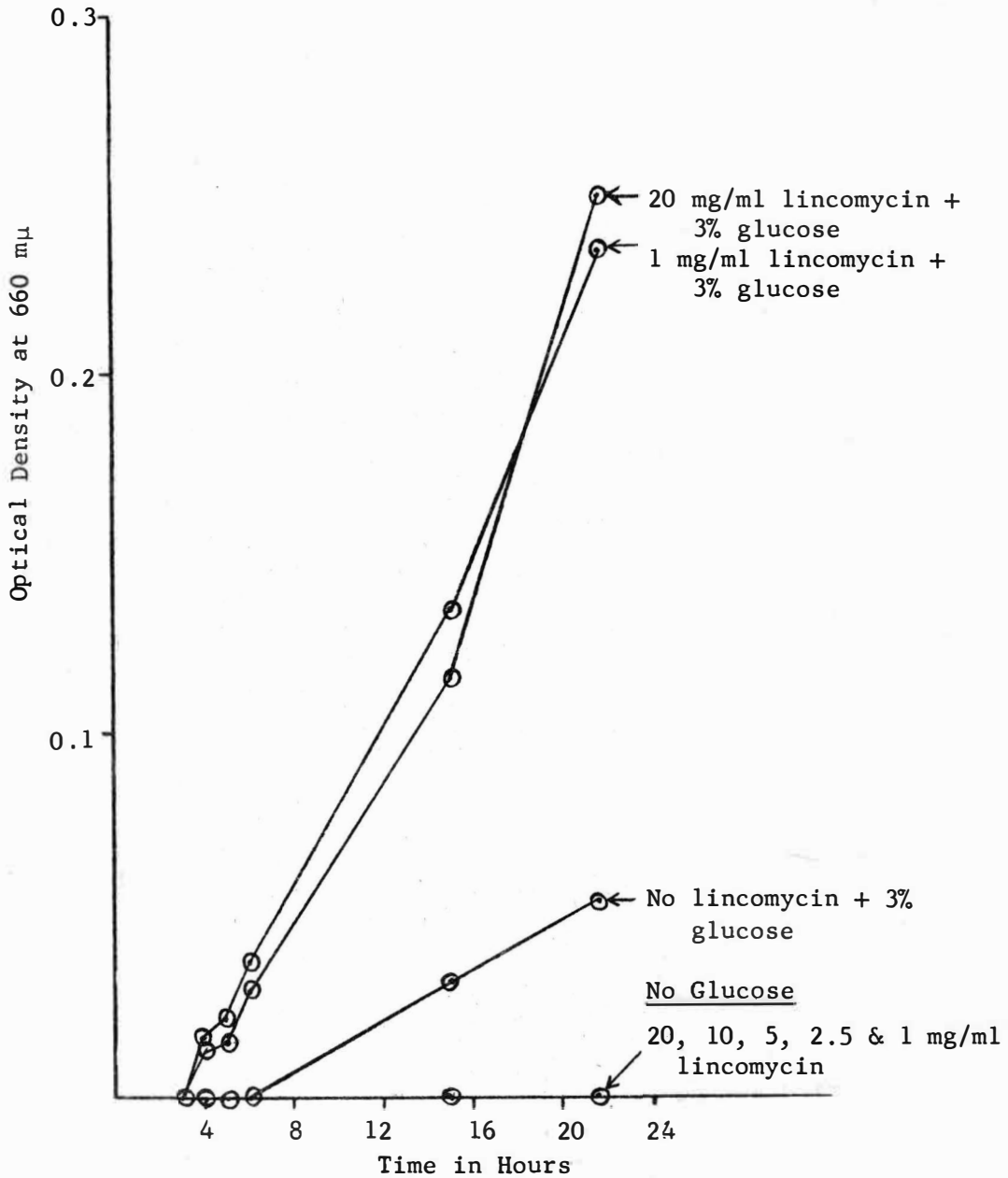

FIGURE 8

Lincomycin Substitution for Biotin and/or Dethiobiotin Saccharomyces sp.
Synthetic Medium pH 6.8 30°C No Aeration

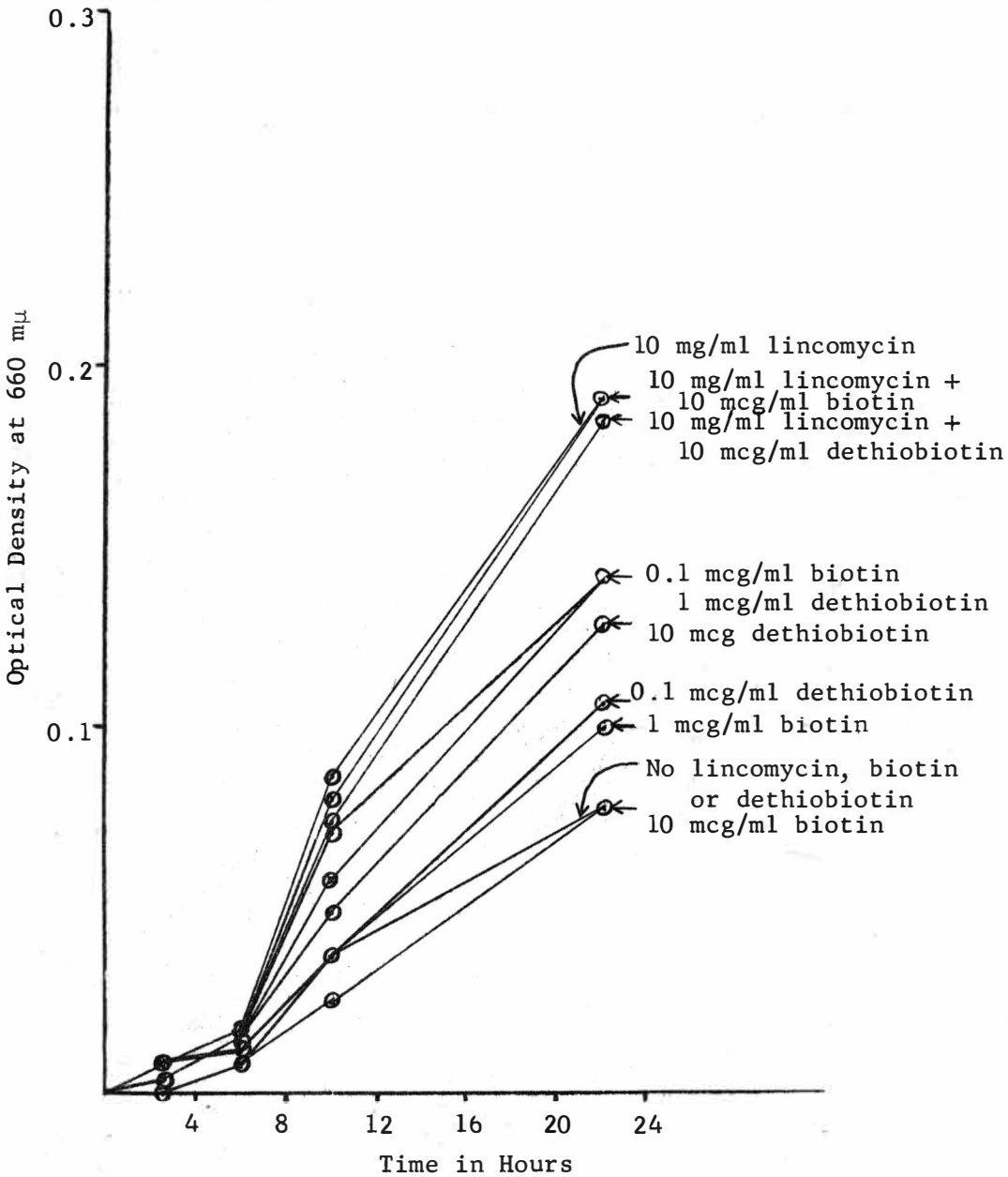

FIGURE 9

Lincomycin Substitution for Thiamine and Lipoic Acid Saccharomyces sp.
Synthetic Medium pH 6.8 30°C No Aeration

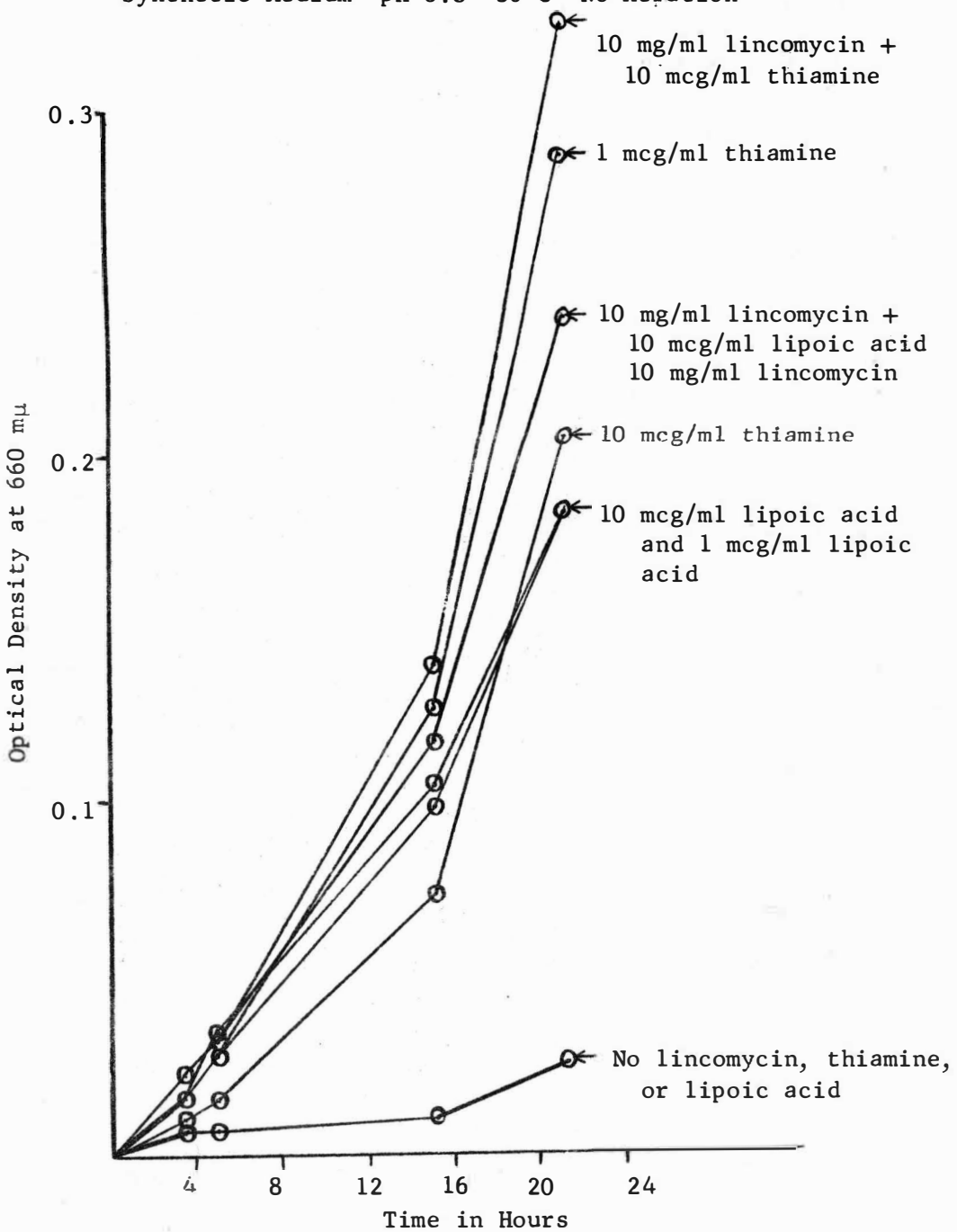

TABLE 10

Vitamins Added to Basal Medium Final Concentration in mcg/ml

Vitamin	mcg/ml in the Test Medium
Biotin	1
Choline	1
Folic Acid	1
Nicotinamide	1
Pantothenic Acid	1
Pyridoxal	1
Thiamine	1
Riboflavin	0.1
Inositol	0.54

FIGURE 10

Saccharomyces sp. Growth Response to Lincomycin in Synthetic Medium + Vitamins pH 6.8 30°C No Aeration

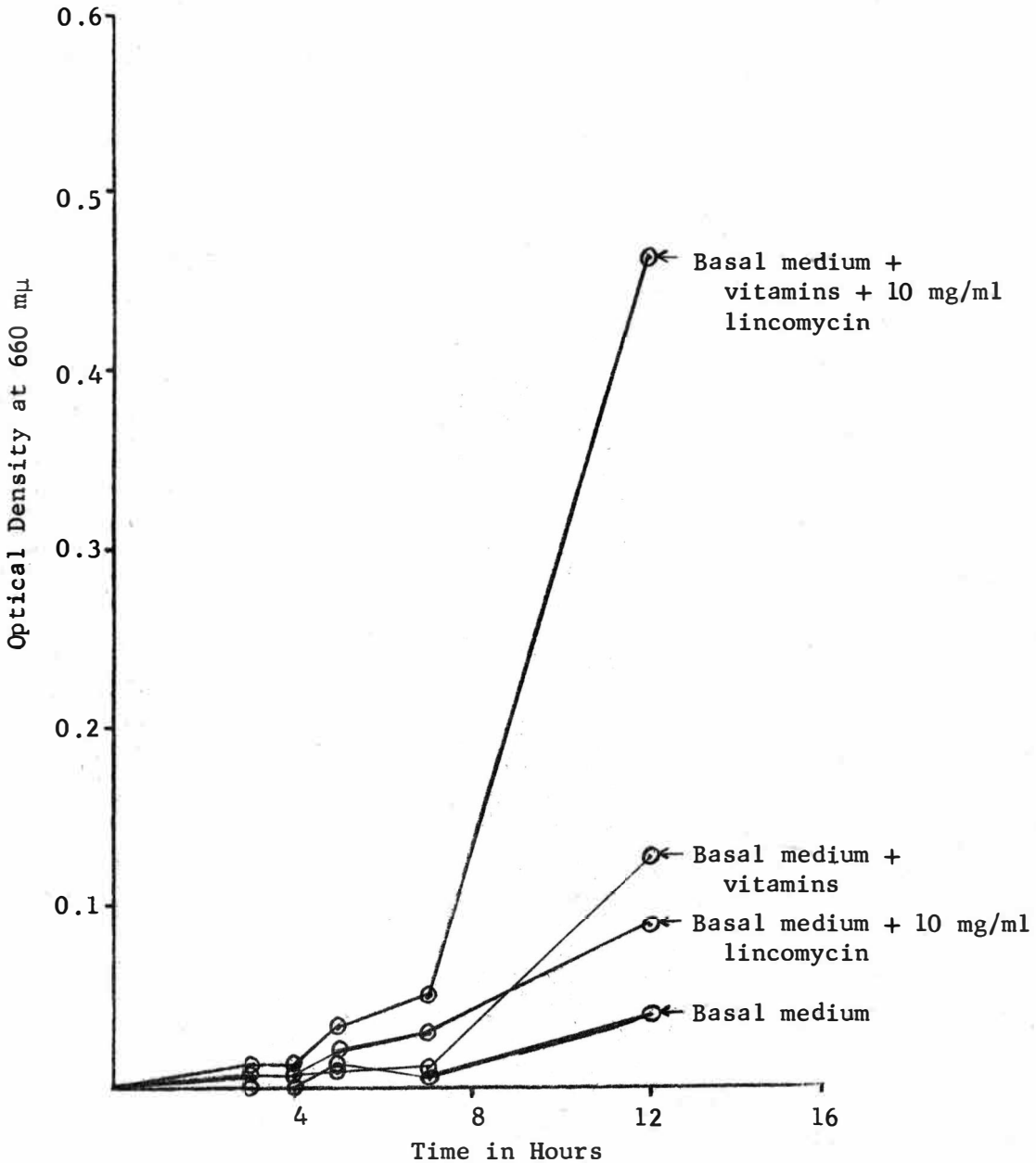

FIGURE 11

Lincomycin Relation to Amino Acid Metabolism Saccharomyces sp.
Synthetic Medium pH 6.8 30°C No Aeration

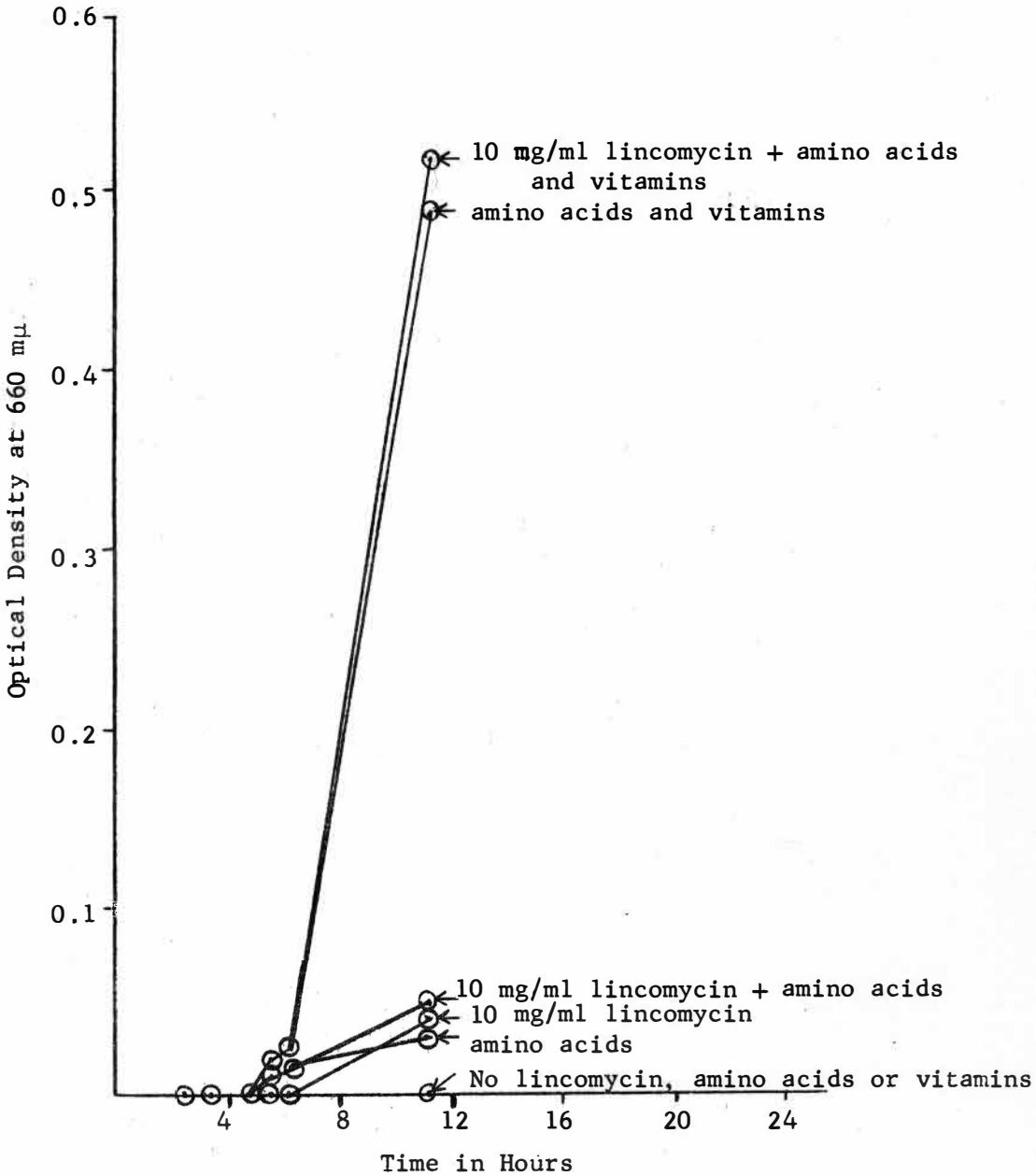

TABLE 11

Amino Acids Added to Basal Medium Final Concentration in mcg/ml

Amino Acid	mcg/ml in the Test Medium
Arginine	21
Cystine	12
Histidine	8
Isoleucine	26
Leucine	26
Lysine	26
Methionine	8
Phenylalanine	16
Threonine	24
Tryptophane	4
Tyrosine	18
Valine	24
Glutamine	300

FIGURE 12

Lincomycin Relation to Nucleotide Metabolism *Saccharomyces* sp.
Synthetic Medium pH 6.8 30°C No Aeration

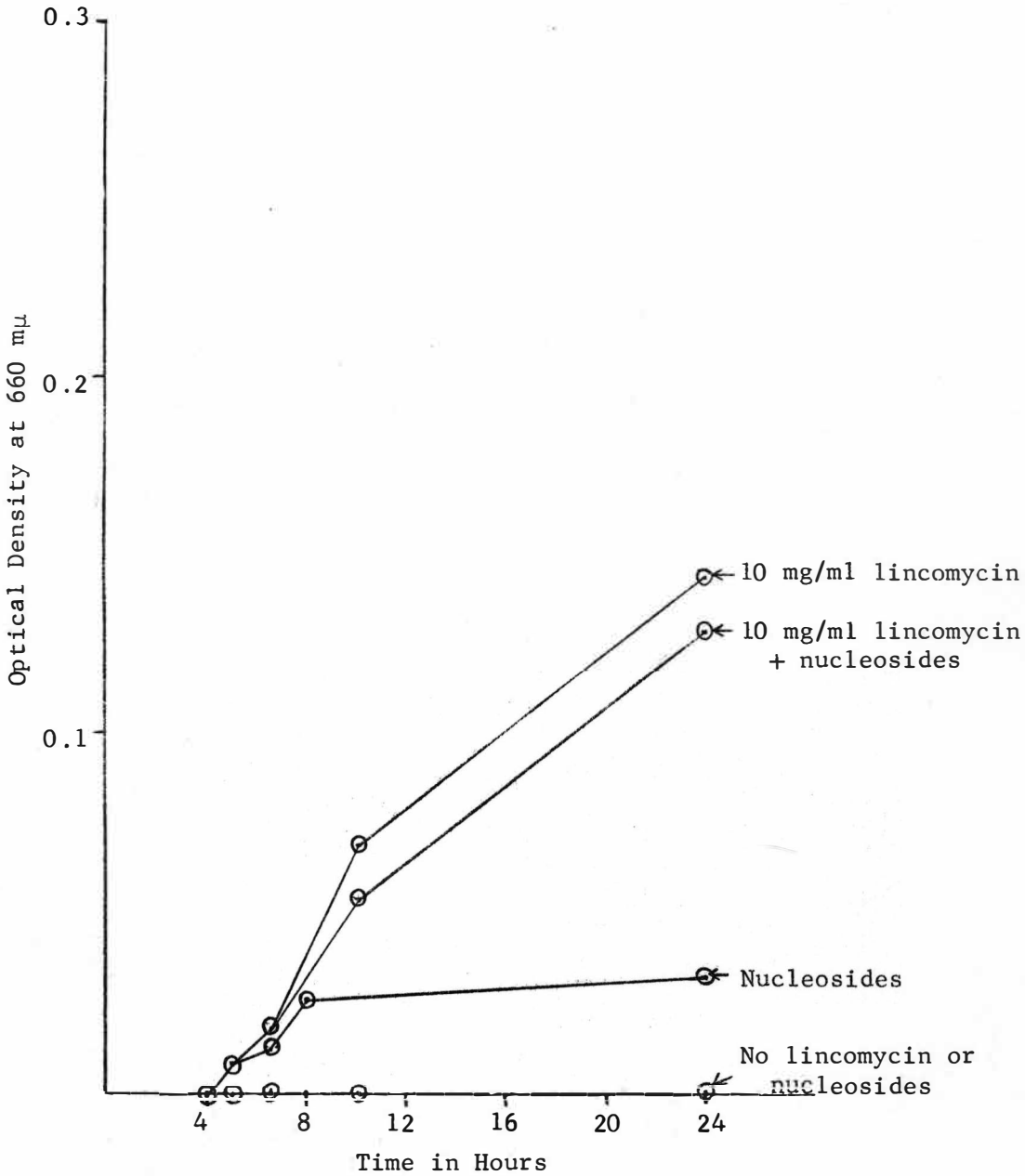

TABLE 12

Nucleosides Added to Basal Medium Final Concentration in mcg/ml

Nucleoside	mcg/ml in the Test Medium
Adenosine	10
Guanosine	10
Thymidine	10
Cytidine	10
Uridine	10

TABLE 13

The Effect of Equal Concentrations of Lincomycin, Compound I and Compound II on Yeast Growth in Synthetic Medium and Vitamins pH 6.8[1]
O.D. at 660 mμ

mg/ml	0 Hr. No Air	5 Hr. No Air	11 Hr. No Air
Lincomycin			
29.45	.000	.027	.284
14.73	.000	.019	.287
7.36	.000	.023	.274
3.68	.000	.013	.252
1.84	.000	.019	.225
No Lincomycin	.000	.009	.028
Compound I			
14.80	.000	.025	.336
7.40	.000	.005	.296
3.70	.000	.014	.330
1.85	.000	.029	.371
0.93	.000	.029	.355
No Compound I	.000	.009	.027
Compound II			
16.96	.000	.000	.008
8.43	.000	.000	.053
4.22	.000	.000	.155
2.11	.000	.009	.288
1.05	.000	.013	.409
No Compound II	.000	.000	.244
Compound I & II			
31.76	.000	.038	.345
15.83	.000	.032	.420
7.92	.000	.050	.495
3.96	.000	.009	.357
1.98	.000	.041	.398
No Compound I or II	.000	.009	.187

1 = Temp. incub. = 30°C.

FIGURE 13

Compound I and II Utilization as a Substrate Test Organism = Saccharomyces sp. Synthetic Medium + Vitamins pH 6.8 30°C
No Aeration

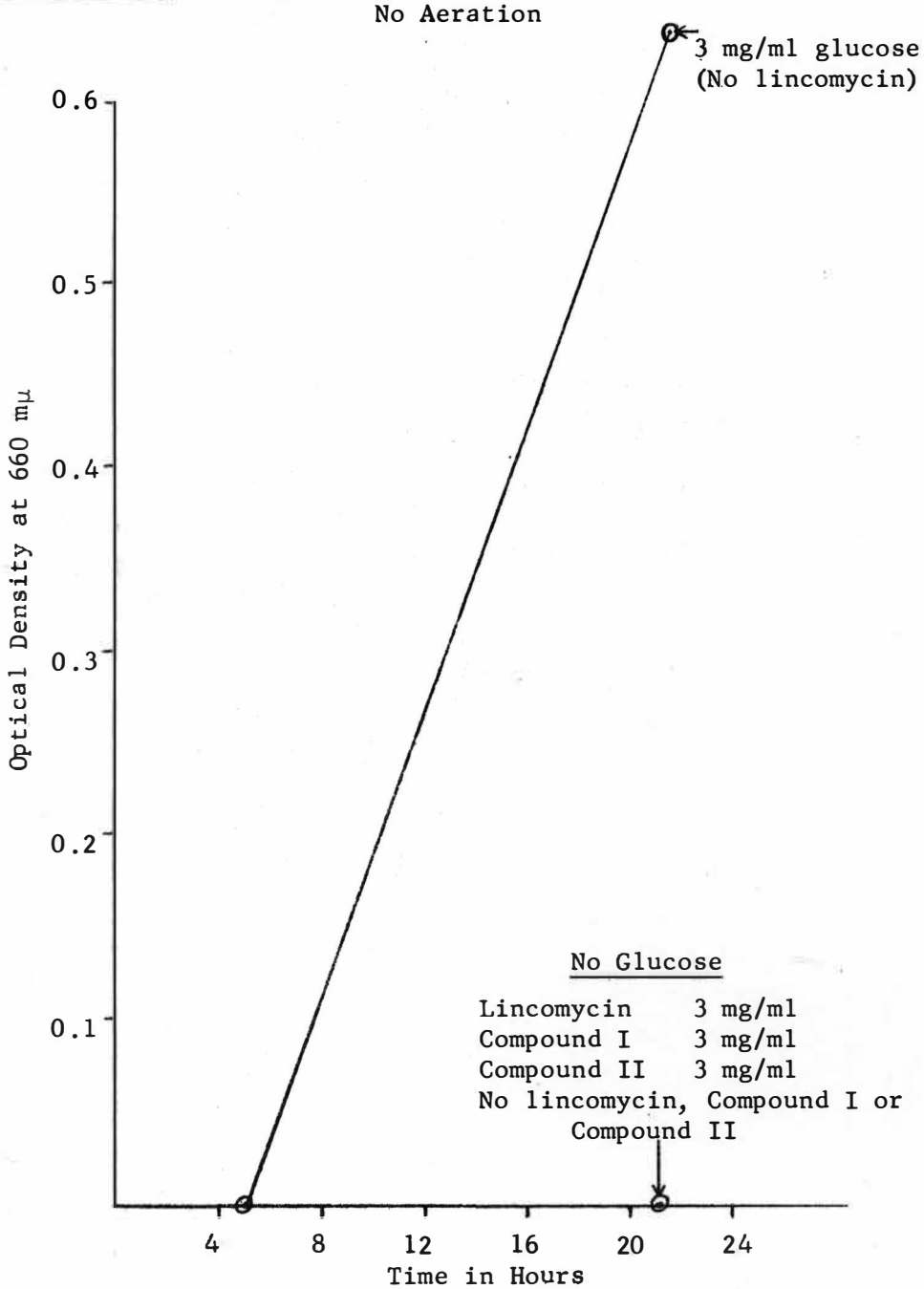

TABLE 14

Candida albicans Stimulation by Lincomycin in Synthetic Medium pH 6.8 + Vitamins[1]

Conc. of Lincomycin/ml	0 Hr.	12 Hr.
10 mg	.000	.339
5 mg	.000	.306
2.5 mg	.000	.306
1 mg	.000	.263
Growth Control (No Lincomycin)	.000	.178

1 = Optical density measurements taken in Beckman Model B Spectrophotometer at 660 mμ. Cells were grown at 30°C - no aeration.