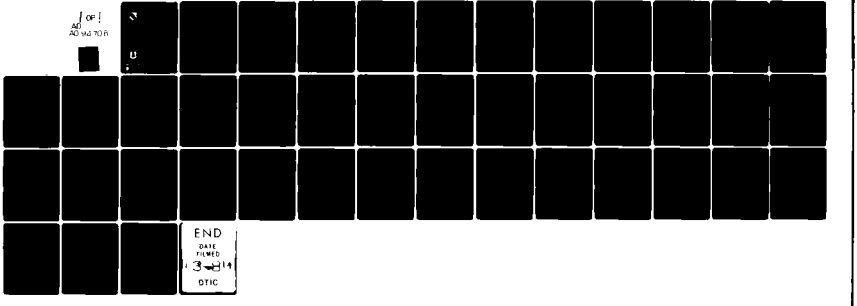
AD-A094 706 FRANK J SEILER RESEARCH LAB UNITED STATES AIR FORCE A--ETC F/G 6/5
THE EFFECT OF HYPERBARIC OXYGEN ON CERTAIN GROWTH FEATURES OF F--ETC(U)
DEC 80 W J CAIRNEY
UNCLASSIFIED FJSRL-TR-80-005 NL



LEVEL II

(2)

FRANK J. SEILER RESEARCH LABORATORY

FJSRL TECHNICAL REPORT 80-005

DECEMBER 1980

THE EFFECT OF HYPERBARIC OXYGEN ON CERTAIN GROWTH FEATURES OF FOUR DERMATOPHYTES

BY

WILLIAM J. CAIRNEY

PROJECT 2303

AIR FORCE SYSTEMS COMMAND

UNITED STATES AIR FORCE

FJSRL-TR-80-005

This document was prepared by the Faculty Research Division, Directorate of Chemical Sciences, Frank J. Seiler Research Laboratory, United States Air Force Academy, Colorado. The research was conducted under Project Work Unit Number 2303-F1-33, "The Effect of Hyperbaric Oxygen on Certain Growth Features of Four Dermatophytes." Major William J. Cairney was the Project Scientist in charge of the work.

When US Government drawings, specifications or other data are used for any purpose other than a definitely related Government procurement operation, the Government thereby incurs no responsibility nor any obligation whatsoever, and the fact that the Government may have formulated, furnished or in any way supplied the said drawings, specifications or other data is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation or conveying any rights or permission to manufacture, use or sell any patented invention that may in any way be related thereto.

Inquiries concerning the technical content of this document should be addressed to the Frank J. Seiler Research Laboratory (AFSC), FJSRL/NC, USAF Academy, Colorado 80840. Phone AC 303-472-2655.

This report has been reviewed by the Chief Scientist and is releasable to the National Technical Information Service (NTIS). At NTIS it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

William J. Cairney

WILLIAM J. CAIRNEY, Major, USAF, BSC
Project Scientist
Director of Research/DFB

Kenneth E. Siegenthaler

KENNETH E. SIEGENTHALER, Lt Col, USAF
Director
Directorate of Chemical Sciences

William D. Siuru, Jr.

WILLIAM D. SIURU, Jr., Lt Col, USAF
Commander

Copies of this report should not be returned unless return is required by security considerations, contractual obligations, or notice on a specific document.

Printed in the United States of America. Qualified requestors may obtain additional copies from the Defence Documentation Center. All others should apply to: National Technical Information Service
5285 Port Royal Road
Springfield, Virginia 22161

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: FJSRL-TR-80-005	2. GOVT ACCESSION NO.: ADA094706
3. RECIPIENT'S CATALOG NUMBER:	
4. TITLE (and Subtitle): The Effect of Hyperbaric Oxygen on Certain Growth Features of Four Dermatophytes.	5. TYPE OF REPORT & PERIOD COVERED: Summary Report.
	6. PERFORMING ORG. REPORT NUMBER:
7. AUTHOR(s): William J. Cairney, Major, USAF, BSC, PhD	8. CONTRACT OR GRANT NUMBER(s):
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Department of Biology, DFB, USAF Academy, Colorado 80840	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: 2303-F1-33
11. CONTROLLING OFFICE NAME AND ADDRESS: Frank J. Seiler Research Laboratory (AFSC), FJSRL/NC, USAF Academy, Colorado 80840	12. REPORT DATE: December 1980
	13. NUMBER OF PAGES: 37
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)	15. SECURITY CLASS. (of this report): Unclassified
	15a. DECLASSIFICATION DOWNGRADING SCHEDULE: N/A

16. DISTRIBUTION STATEMENT (of this Report)
Approved for Public Release;
Distribution Unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Compression Chamber	Hyperbaric Oxygenation	Microsporum gypseum
Compression Therapy	Hyperbarics	Trichophyton mentagrophytes
Dermatophyte	Medical Mycology	Trichophyton tonsurans
Hyperbaric	Microsporum canis	Oxygen therapy

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Four dermatophyte fungi, Trichophyton tonsurans, Trichophyton mentagrophytes, Microsporum gypseum, and Microsporum canis, isolated from human and animal tinea infections, were observed in vitro for macroscopic and microscopic growth characteristics at varying hyperbaric oxygen levels, and for various exposure times and intervals. All organisms were completely inhibited in oxygen at 3 atmospheres absolute. Trichophyton mentagrophytes, Microsporum gypseum, and Microsporum canis were completely inhibited for the duration of exposure to

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

hyperbaric oxygen according to treatment tables routinely used for patients with Clostridial gas gangrene. Trichophyton tonsurans was inhibited only slightly during treatment table exposures. Overall experimental results provide a partial base for use of compression chamber therapy in treatment of tinea infections.

FJSRL-TR-80-005

THE EFFECT OF HYPERBARIC OXYGEN ON CERTAIN GROWTH FEATURES OF FOUR DERMATOPHYTES

By

Major William J. Cairney

December 1980

Department of Biology
US Air Force Academy, Colorado 80840

Table of Contents

INTRODUCTION

Although oxygen is supportive of the vast majority of life forms, every organism functions optimally within only a certain range of oxygen pressure. Metabolic processes are inhibited for most organisms when oxygen is not present in sufficient quantity to be effective as the terminal receptor for electron transport. Above the optimal range, oxygen becomes toxic. The oxygen toxicity limit varies from organism to organism.

Organisms classically considered anaerobes may be inhibited by minute amounts of oxygen. This phenomenon has permitted oxygen under pressure to be used effectively in treating gas gangrene caused by Clostridium spp., especially Clostridium perfringens (2). Organisms classically considered aerobes are also inhibited by oxygen above certain levels which vary from organism to organism. Some organisms have been shown to be very tolerant of high levels of oxygen, and others shown to be inhibited by levels slightly greater than that present in air at sea level (4, 5, 8, 9, 10, 11, 12, 13, 14, 15, and 16).

Some research has been done on the effects of hyperbaric oxygen on various dermatophyte species. Karsner and Saphir in 1926 (10) and Williams in 1938 (16) subjected species of Trichophyton, Microsporum (reported by both as Microsporon), and Epidermophyton to levels of oxygen between 1% and 99.5%, presumably at sea level pressures. Far from being supportive of one another, these reports contradict in a number of critical

areas. Karsner and Saphir state that oxygen concentrations of 76% or greater inhibited most molds in their study, especially the pathogens (a label for which dermatophytes would certainly qualify). Williams reports no inhibition even at 99% for some of the same organisms used by Karsner and Saphir. Sabouraud's medium was used in both studies.

A second problem area (perhaps for both papers) is the inability to verify which dermatophyte organisms were really used. Dermatophyte taxonomy and nomenclature have been the subjects of active debate. Several good reviews of dermatophyte taxonomy and accompanying problems do exist and prospective dermatophyte researchers ought to be acquainted with them. Papers by Ajello (1), Emmons (6), and Georg (7) are particularly valuable.

Georg, with amply documented justification, brings Trichophyton crateriforme, Trichophyton fumatum, and Trichophyton sulfureum into synonymy with Trichophyton tonsurans (7). She also brings Trichophyton gypseum and Trichophyton granulosum into synonymy with Trichophyton mentagrophytes. Karsner and Saphir, and Williams, treated the synonymous organisms as separate organisms and reported different results for their growth in hyperbaric oxygen.

The purpose of the present study was to determine growth rates and observe macroscopic and microscopic appearance of four dermatophyte species when subjected to various hyperbaric oxygen levels.

MATERIALS AND METHODS

Four dermatophytes were chosen on the basis of their being isolated frequently from soil and animal hosts and their being least

ambiguous taxonomically. Organisms selected were Trichophyton tonsurans Malmsten, Trichophyton mentagrophytes (Robin) Blanchard, Microsporum gypseum (Bodin) Guiart & Grigoraki, and Microsporum canis Bodin. Isolates were obtained from the Armed Forces Institute of Pathology and the New York State College of Veterinary Medicine at Cornell University.

Five experiments (excluding repetitions to insure repeatability) were undertaken to determine the effects of hyperbaric oxygen on these dermatophytes. Following a separate study to determine media for most observable and repeatable growth characteristics (3), all organisms were maintained on Sabouraud's dextrose agar in tissue culture flasks. Sabouraud's dextrose agar was also used for all single-spore preparation and growing of fungi exposed to hyperbaric oxygen. All exposures of organisms to hyperbaric oxygen were carried out in a table top (31 liter capacity) hyperbaric chamber made available for the project by the Armed Forces Institute of Pathology.

In all experiments, organisms were compressed to the desired pressure in less than 30 seconds. Following compression, the chamber was flushed rapidly with five chamber volumes of 100% oxygen to insure a pure oxygen environment. Each time the chamber was decompressed for the removal of cultures, this same compression/flushing operation was performed upon continuation of the experiment. During compression periods, a 5 liter/hour flow was maintained through the system.

Organisms to be exposed to oxygen, along with controls, were propagated from single spores poured in water suspensions on Petri

plates containing Sabouraud's dextrose agar. Suspensions were poured 24 hours before plugs containing germinating single spores were cut out and transferred to Sabournaud's dextrose agar plates to be used for the exposures. In all experiments, each plate contained four single spores of a given organism placed on the medium, one in the center of each quadrant. No inhibitors of any kind were used in order to reduce variables. The pH of the medium was 5.6. The room in which the experiments were performed was held at 25° (±1°)C. Cultures were exposed to room fluorescent lighting for the duration of all experiments. The hyperbaric chamber, constructed of transparent plexiglas, allowed exposure of organisms to room light.

In the first experiment, single-spored cultures of all organisms were placed in the hyperbaric chamber and exposed to a steady, 3-ATA (3 atmospheres absolute) level of oxygen. Controls were allowed to grow in air under room pressure. Air pressurized controls (3 ATA) were run at a separate time but under otherwise identical conditions to insure that effects were caused by oxygen rather than by pressure alone. Four 1-ATA air control plates (total of 16 colonies) and four 3-ATA air control plates (16 colonies) were run for each experiment.

Organisms were removed from the chamber at intervals of 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, and 264 hours. At each interval four plates (16 colonies) were removed and surveyed for macroscopic and microscopic growth features. Colony diameters were measured as an index of linear growth. All plates were retained for three weeks from initiation of the experiment and measurements

made of colonies of each plate at the same time each day. Microscopic mounts were made to correlate microscopic development with macroscopic features.

In removing plates for examination, the chamber was depressurized, appropriate plates removed, and the chamber repressurized and flushed. Plates not to be removed were left undisturbed as much as possible, except that where growth was evident in the chamber, plates were removed quickly for measurement and then replaced. The entire depressurization-repressurization-flush procedure took no more than five minutes.

In a second experiment, dermatophyte cultures were exposed to hyperbaric oxygen according to exposure tables used for treating Clostridial gas gangrene (2). Petri plates containing 24-hour single-spored cultures of each organism were prepared in the same manner as for the previous experiment. Eight plates (32 potential colonies) were prepared for chamber exposure for each fungus. Four 1-ATA and four 3-ATA air controls were also made up in a similar fashion for each.

The experiment plates were exposed to 3-ATA oxygen for three 90-minute periods during the first 24 hours, treatments beginning approximately 8 hours apart. In each of two succeeding 24-hour periods, cultures were exposed to the same 3-ATA level for two 90-minute periods, treatments beginning approximately 12 hours apart. Cultures were thus exposed to seven 90-minute periods of oxygen at 3 ATA over a span of three days. Fungi were checked for growth throughout the experiment and measurements made where appropriate. Microscopic features were observed regularly. Again, 3-ATA air controls were run

using identical methods but at another time due to having only one hyperbaric chamber.

Results from these first two experiments suggested that some effect might be realized using the same 3-ATA level but at longer intervals. Two additional experiments were thus performed. In the first, organisms were exposed to the 90-minute, 3-ATA level at 18-hour intervals. In the second, organisms were exposed at 12-hour intervals.

In a final experiment, the dermatophytes were exposed to 1-ATA oxygen to determine the effects of this steady exposure to a lower pressure. Plates were prepared in the same manner as for the 3-ATA experiment. Organisms were observed frequently over a 7-day period. Colonies were measured every 25 hours.

RESULTS

Growth curves for all organisms in the 3-ATA experiment are shown in Figures 5 through 8. Growth curves for the "treatment table" exposures are shown in Figures 9 through 12. Microscopic features of Trichophyton tonsurans, Trichophyton mentagrophytes, Microsporum gypseum, and Microsporum canis are illustrated in Figures 1, 2, 3, and 4, respectively. Time intervals expressed in Figures 1 through 4 are for controls only because comparison is made directly in the text of how exposed organisms match the controls at given times. In all graphs, "controls" are marked "c"; "e" refers to "exposed" plates. The number following "e" indicates the hour interval at which that set of plates was removed from the chamber.

Data from these experiments confirm the general results of the Williams work (16), and contradict the Karsner and Saphir (10)

results. All fungi were inhibited at 3 ATA and no development was seen as long as that pressure was maintained. Following removal from the chamber, all organisms except Trichophyton tonsurans grew at a rate identical to the controls following a certain "lag" period. This "lag" period was also reported by Robb and Caldwell in separate studies on the effects of hyperbaric oxygen on organisms other than ones considered in this study (4, 5, 13).

This author proposes that the "lag" period be defined as the time it takes for an exposed isolate, following removal from the chamber, to reach a given point on the growth curve, minus the time taken by the corresponding control to reach the same point (3). Thus, for Microsporum gypseum in the 3-ATA experiment, the control reached a 2 mm diameter by hour 58. Plates exposed for 48 hours reached a 2 mm diameter by hour 115. Given the 48-hour period of no growth in the chamber and subsequent 67 hours to produce a 2 mm colony, the lag time would equal 9 hours.

This definition perhaps has some limitations, but if a low growth curve point is selected, a decent approximation of lag time can be achieved. It is difficult without a constantly observed slide mount to determine the exact moment of resumption of growth. If too high a point (>5 mm) is selected as a reference point, the slope of the growth curve would affect the calculation.

For both Microsporum species at 3 ATA, growth on the e24 plates was enhanced after this relatively short exposure, but increasing the time in the chamber increased the lag time greatly, up to the range of 40+ hours. T. mentagrophytes was the most sensitive fungus, a lag time being evident in even the e24 colonies.

72-hour plates showed a lag period of almost 40 hours. Significant also is that T. mentagrophytes cultures kept in the chamber for 96 or more hours never resume growth following removal.

T. tonsurans was inhibited as long as it was kept under 3-ATA oxygen, but beginning growth was erratic following removal. In some cases, initial growth seems to have been enhanced by the oxygen, although overall slopes of T. tonsurans growth curves approximated those of controls.

No previous researcher has attempted to correlate microscopic and macroscopic features of fungal development under hyperbaric oxygen conditions. While some fungi would perhaps show effects at the microscopic level, all of these dermatophytes progressed uneventfully after oxygen exposure was terminated, with the exception of the e96 and e120 cultures of T. mentagrophytes which did not grow at all. Once growth began after any given interval there was no detectable difference between exposure cultures and controls in the development of macroconidia, microconidia, or hyphal structures with time. Pigmentation appeared unaltered and there was no difference in either size or abundance of conidia. Unpublished data from this laboratory show that significant cytological changes occur in other fungi exposed to 3-ATA oxygen. These fungi include Mucor sp. and Candida albicans.

No previous worker has reported subjecting dermatophytes to treatment table exposures. In the cases of M. gypseum, M. canis, and T. mentagrophytes, treatment table exposures resulted in a cessation of growth. Trichophyton tonsurans showed retardation but not complete inhibition. T. tonsurans was growing while treatments were in progress.

Exposing the organisms to 90-minute periods of 3-ATA oxygen at 18-hour and 12-hour intervals resulted in no distinguishable differences between exposed plates and controls. In some cultures, exposed organisms seemed to be somewhat retarded but statistical analysis showed these differences not to be significant. There were no differences in microscopic features between exposed fungi and controls.

Exposure to 1-ATA oxygen likewise resulted in no significant differences in growth rates. Microscopic characteristics were likewise unchanged. This result is not consistent with Karsner's and Saphir's report of inhibition of pathogens (including some of the same genera studied here) at oxygen concentrations higher than 76% (at 1 ATA).

DISCUSSION

The major question remaining is whether adequate in vitro evidence now exists to suggest that hyperbaric oxygen might be effective in treating dermatomycoses caused by these organisms. Such evidence for Trichophyton tonsurans seems marginal. For the other fungi, however, such basis appears confirmed. The organisms are at least retarded by oxygen levels easily tolerated by humans, and, in the case of T. mentagrophytes, an apparent time tolerance limit for 3-ATA oxygen has been established.

The motive behind the 90-minute exposures at 18-hour and 12-hour intervals was that these dermatophytes might be inhibited completely if re-exposed within lag periods calculated on the basis of the 3-ATA oxygen steady exposure tests. The lack of effect

indicates that an exposure time of more than 90 minutes might be necessary to establish a lag period.

The inhibition seen in three of four cases in the "treatment table" exposures further encourages the idea that compression therapy might be successful in treating tinea infections. The effect of the hyperbaric oxygen might be enhanced if systemic antifungal agents were administered between oxygen exposures. Recent unpublished data from this laboratory indicate that Amphotericin B and perhaps other systemic antifungal agents may be degraded by hyperbaric oxygen (3). Were this to be confirmed, there would be little use in combining these chemotherapeutic agents with hyperbaric oxygen in hopes of getting an additive effect.

Follow-on in vivo studies using methods designed by Krieg et at. (11) would generate additional necessary information including whether or not oxygen would perfuse highly keratinized tissues to a great enough extent over a 90-minute period to make hyperbaric oxygen therapy practical. In fact, studies on diffusion rates in and out of keratinized tissues at these pressures would be necessary to establish a complete evidential basis for anticipated success.

ACKNOWLEDGEMENTS

The author gratefully acknowledges the support of Dr. Richard P. Korf, Professor of Mycology, Dr. Carl W. Boothroyd, Professor of Plant Pathology, and Dr. John Bentinck-Smith, Professor of Clinical Pathology, all of Cornell University, for critical review of the manuscript. The support of Dr. John H. Wolcott and Dr. Richard E. Krieg of the Armed Forces Institute of Pathology was invaluable in providing cultures and equipment. Portions of this paper represent

part of a thesis presented to the Cornell University Graduate School for the degree of Doctor of Philosophy. An active program on hyperbarics and mycotic diseases is presently on-going in the Department of Biology, United States Air Force Academy. The author appreciates the support of the Frank J. Seiler Research Laboratory in sponsoring this effort.

Note: Reference cultures (dried) of Trichophyton tonsurans, Trichophyton mentagrophytes, Microsporum gypseum, and Microsporum canis are on file in the Plant Pathology Herbarium at Cornell University. CUP reference numbers are 54847, 54848, 54849, and 54850, respectively.

REFERENCES

1. Ajello, L. 1967. A taxonomic review of the dermatophytes and related species. Sabouraudia 6: 147-159.

2. Bassett, B. E. 1970. Notes from compression chamber team training course. USAF School of Aerospace Medicine. San Antonio.

3. Cairney, W. J. 1977. Developmental effects of hyperbaric oxygen on selected human pathogenic fungi in culture. Ph.D. Thesis. Cornell University. Chaps. 2 and 4.

4. Caldwell, J. 1963. Effects of high partial pressures of oxygen on fungi. Nature, London 197: 772-774.

5. Caldwell, J. 1965. Effects of high partial pressures of oxygen on fungi and bacteria. Nature, London 206: 321.

6. Emmons, C. W. 1934. Dermatophytes. Natural grouping based on the form of the spores and accessory organs. Arch. Derm. Syph. 30: 337-362.

7. Georg, L. K. 1956. Studies on Trichophyton tonsurans. I. The taxonomy of T. tonsurans. Mycologia 48: 65-82.

8. Gifford, G. D. and G. G. Pritchard. 1969. Toxicity of hyperbaric oxygen to yeasts displaying periodic enzyme synthesis. J. Gen. Microbiol. 56: 143-149.

9. Gottlieb, S. F. 1971. Effect of hyperbaric oxygen on microorganisms. Ann. Rev. Microbiol. 25: 111-152.

10. Karsner, H. T. and O. Saphir. 1926. Influence of high partial pressures of oxygen on the growth of certain molds. J. Inf. Dis. 39: 231-236.

11. Krieg, R. E., J. H. Wolcott, and A. Confer. 1975. Treatment of Mycobacterium ulcerans infection by hyperbaric oxygenation. Aviat. Space Environ. Med. 46: 1241-1245.

12. McAllister, T. A., J. M. Stark, J. N. Norman, and R. M. Ross. 1963. Inhibitory effects of hyperbaric oxygen on bacteria and fungi. Lancet 2: 1040-1042.

13. Robb, S. M. 1966. Reactions of fungi to exposure to 10 atmospheres pressure of oxygen. J. Gen. Microbiol. 45: 17-29.

14. Stuart, B., R. Gerschman, and J. N. Stannard. 1962. Effect of high oxygen tension on potassium retentivity and colony formation of bakers' yeast. J. Gen Physiol. 45: 1019-1030.

15. Webley, D. M. 1954. The effect of oxygen on the growth and metabolism of the aerobic thermophilic Actinomycete Micromonospora vulgaris. J. Gen. Microbiol. 11: 114-122.

16. Williams, J. W. 1938. Difference in growth of pathogenic fungi with variation of medium and oxygen tension. J. Lab. Clin. Med. 24: 39-43.

Figure 1. Trichophyton tonsurans

Top Frame - Hyphal growth, day 3.

Middle Frame - Hyphae with occasional microconidia, day 5.

Lower Frame - Microconidia and chlamydospores, day 8.

Time designations are for controls.

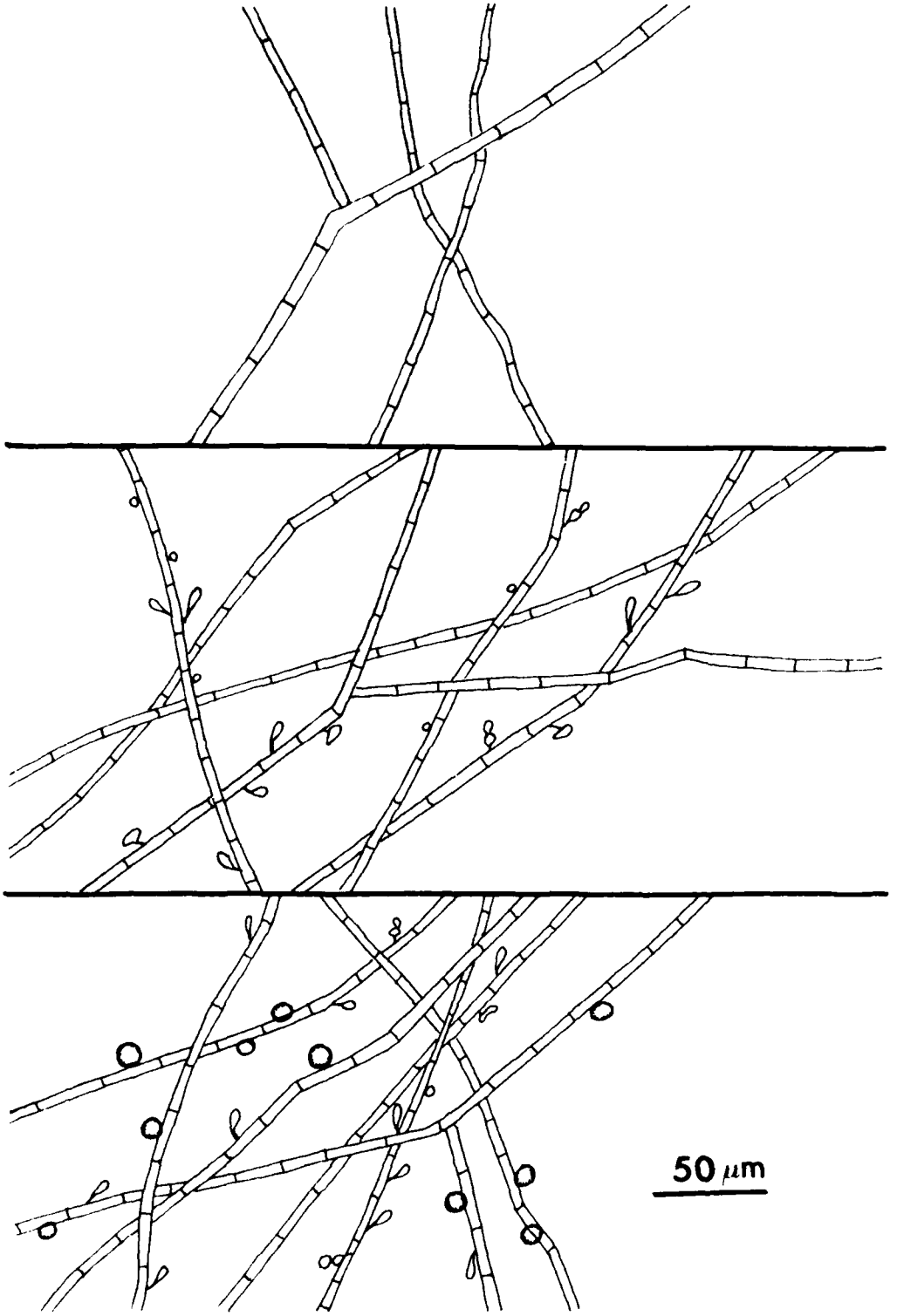

50 μm

Figure 2. Trichophyton mentagrophytes

Top Frame - Appearance of hyphae, day 3.

Middle Frame - Coiled hyphae and oval to pyriform microconidia borne directly on hyphae or singly or in groups on short conidiophores, day 8.

Lower Frame - Chlamydospores and macroconidia, day 14.

Time designations are for controls.

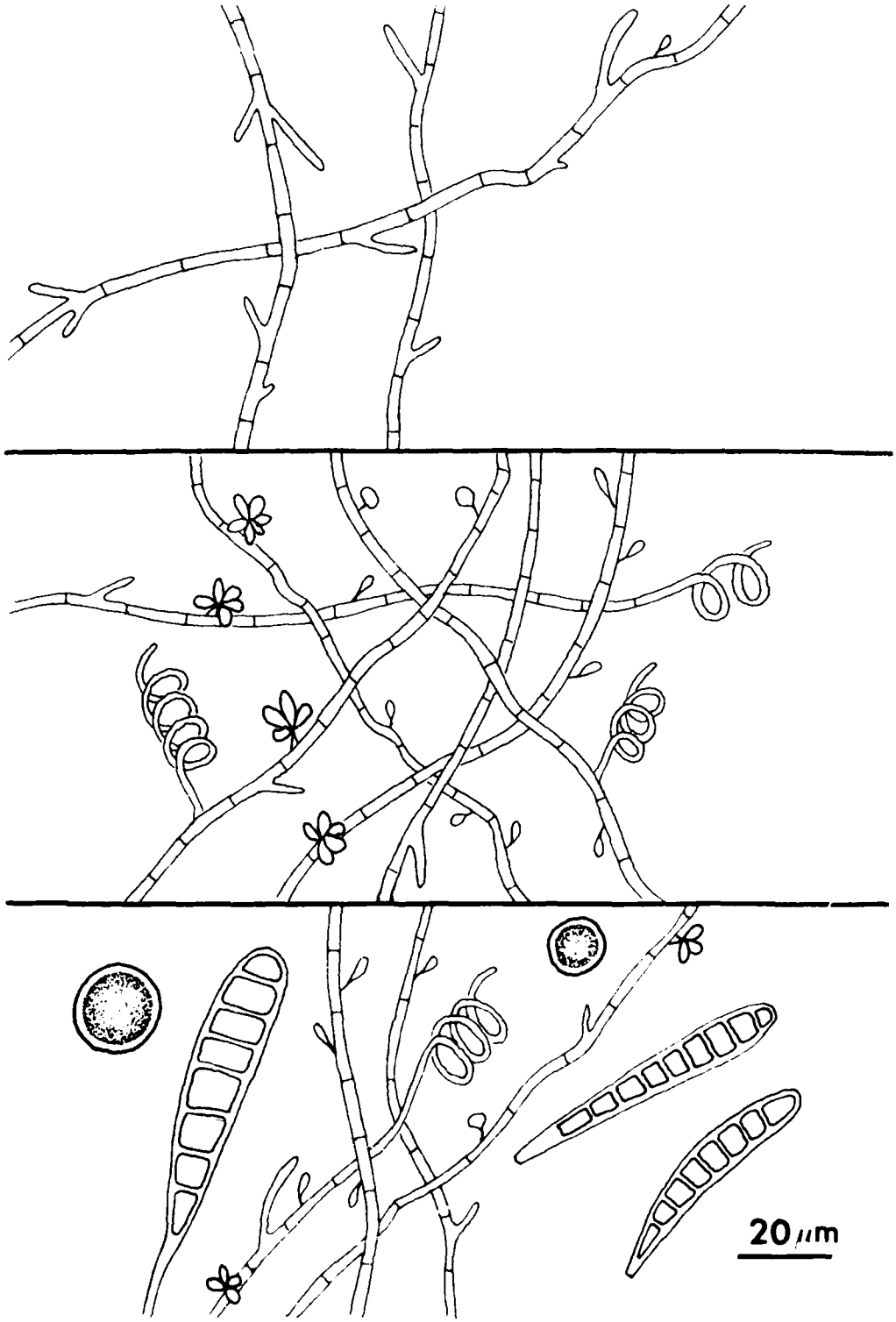

20 µm

Figure 3. Microsporum gypseum

Upper Frame - Hyphae and oval, echinulate macroconidia, day 7.

Lower Frame - Appearance of culture at day 14 showing occasional clavate microconidia borne singly on hyphae.

Time designations are for controls.

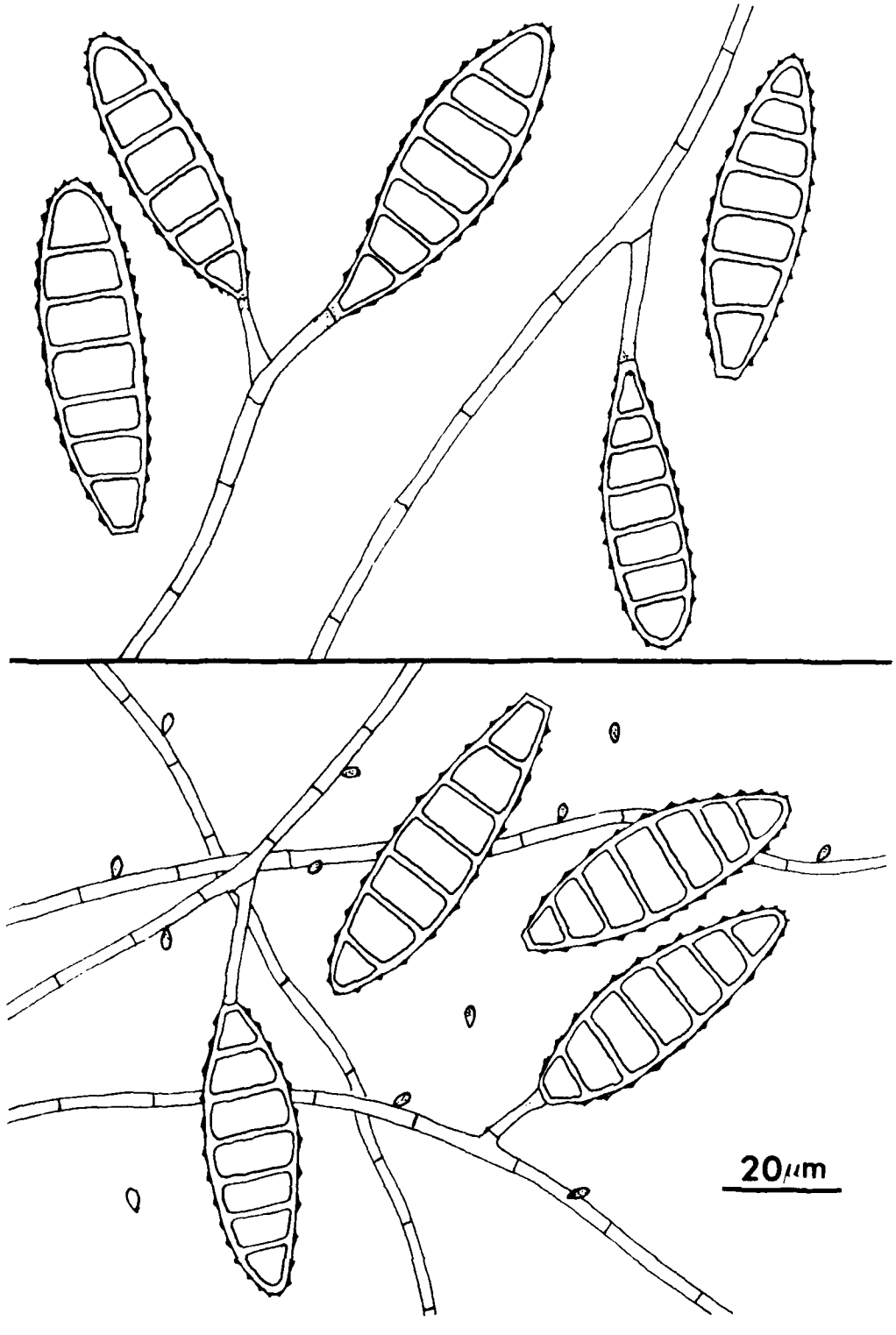

20μm

Figure 4. Microsporum canis

Upper Frame - Hyphae and spindle-shaped, echinulate macroconidia, day 7.

Lower Frame - Appearance of M. canis at day 14 showing occasional sherical to clavate microconidia borne on short conidiophores or directly on hyphae.

Time designations are for controls.

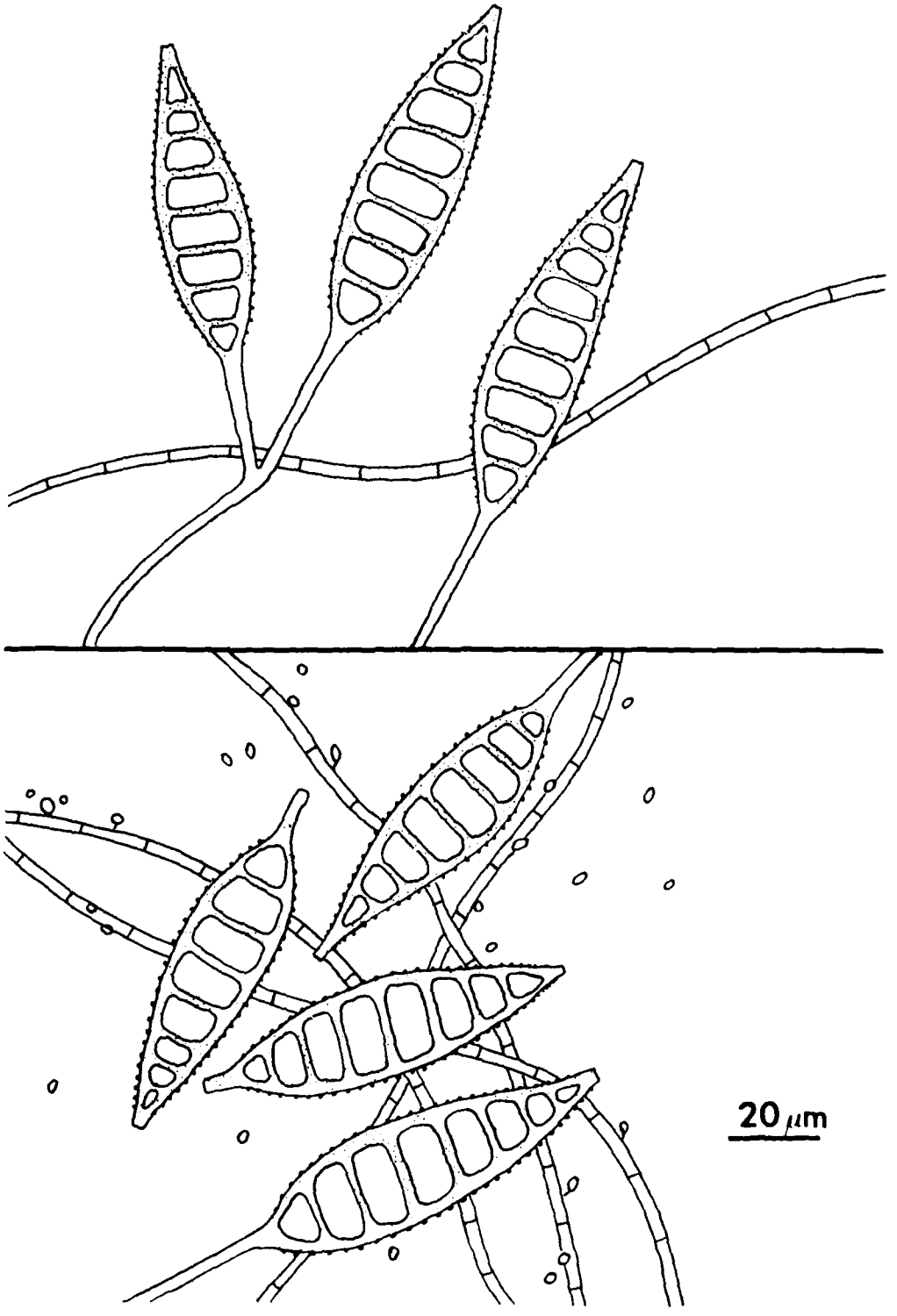

20 µm

Figure 5. Growth curves for cultures of Trichophyton tonsurans exposed to 3-ATA oxygen for indicated intervals.

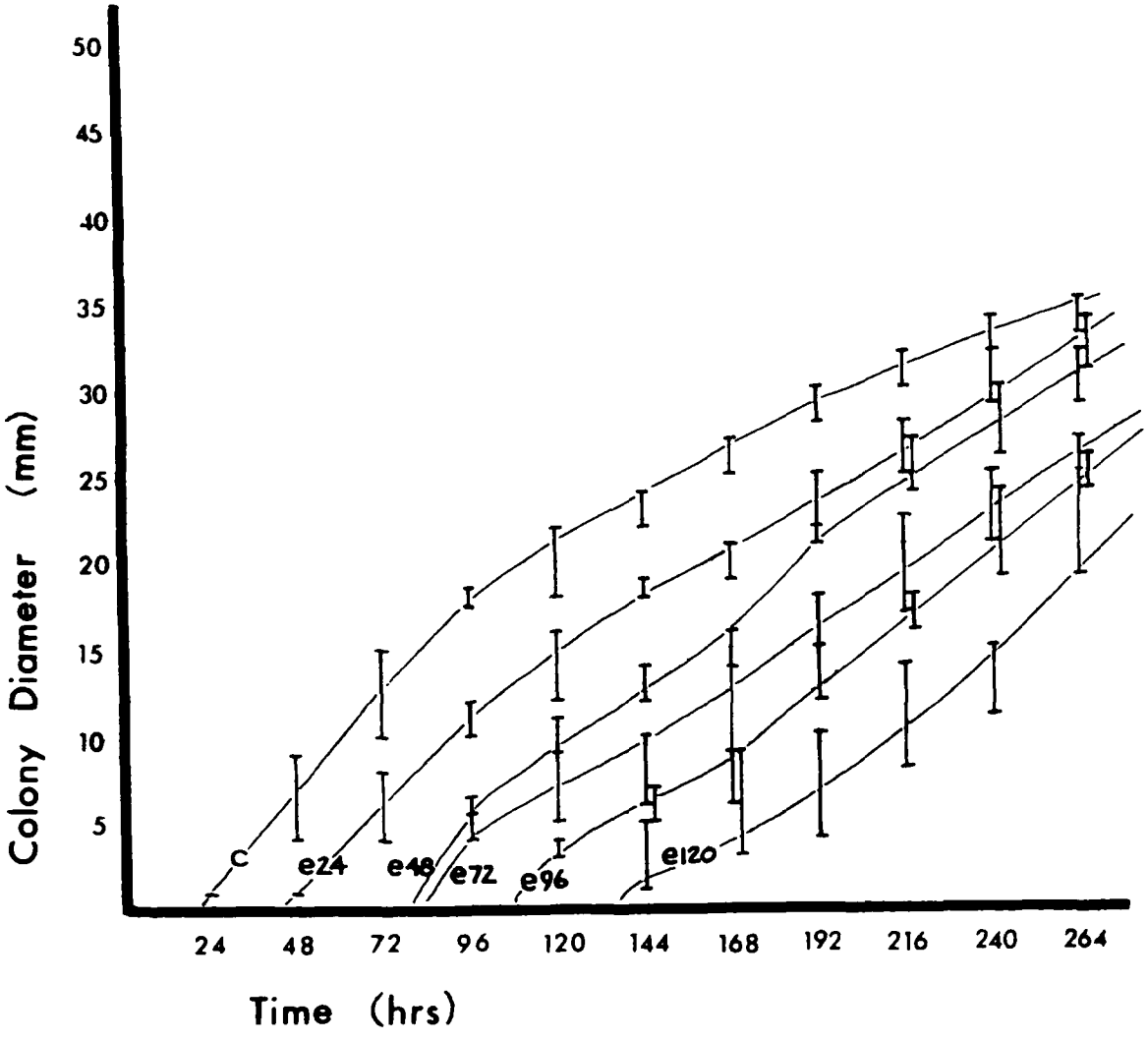

Colony Diameter (mm)
50
45
40
35
30
25
20
15
10
5
C
e24
e48
e72
e96
e120
24
48
72
96
120
144
168
192
216
240
264
Time (hrs)

Figure 6. Growth curves for cultures of Trichophyton mentagrophytes exposed to 3-ATA oxygen for indicated intervals.

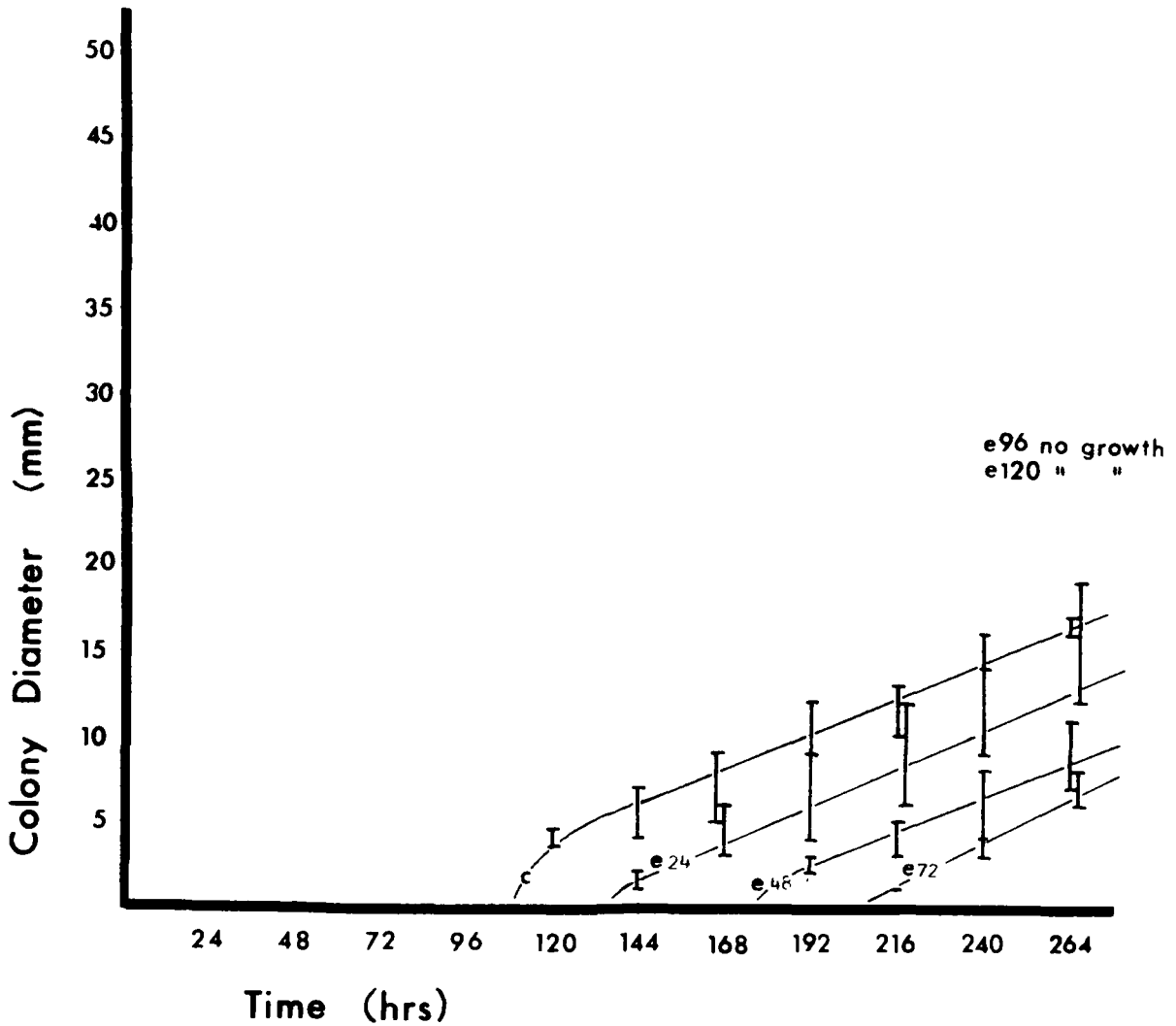

50
45
40
35
30
25
20
15
10
5
Colony Diameter (mm)
e96 no growth
e120 " "
c
e24
e48
e72
24
48
72
96
120
144
168
192
216
240
264
Time (hrs)

Figure 7. Growth curves for cultures of Microsporum gypseum exposed to 3-ATA oxygen for indicated intervals.

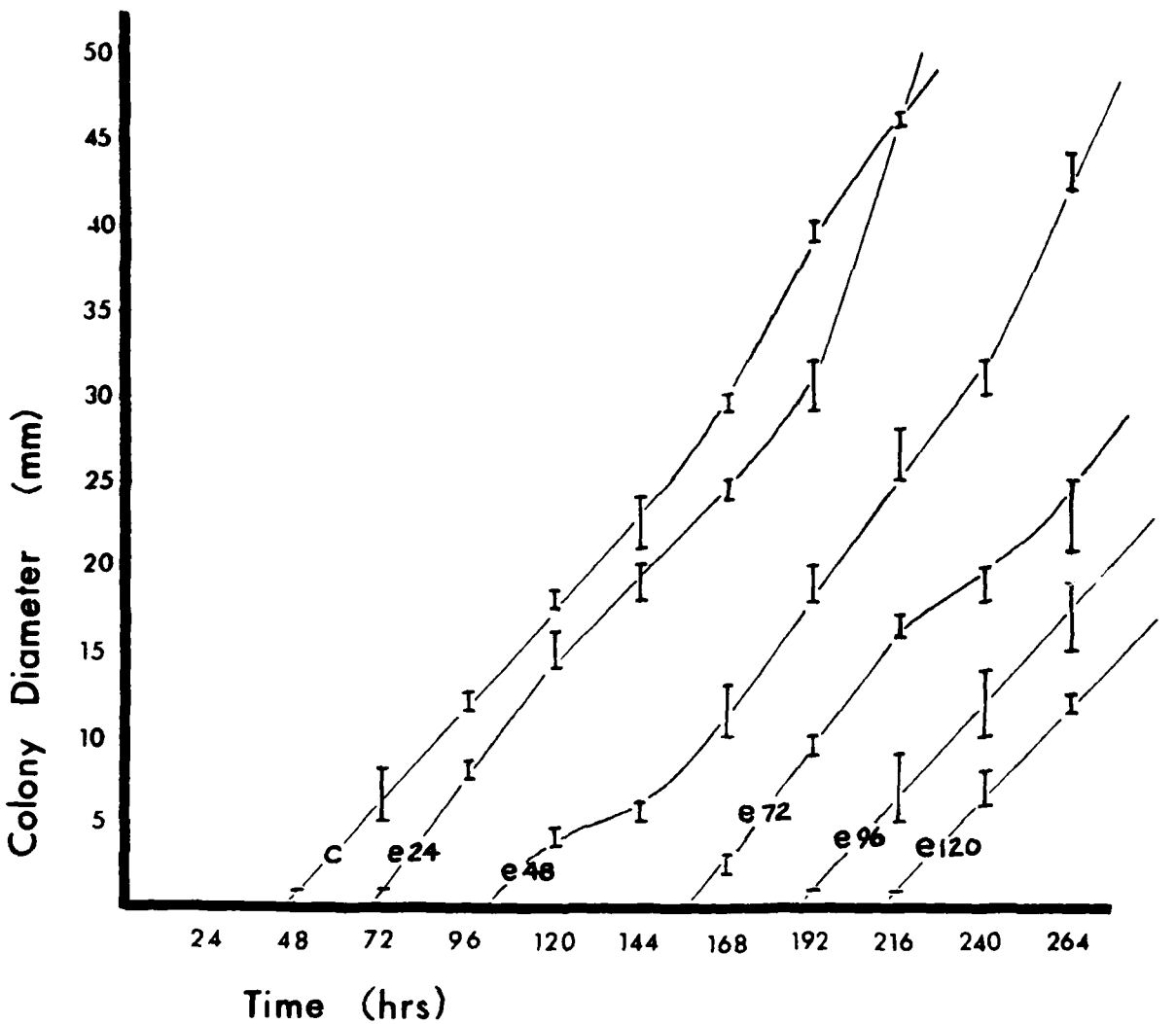

Colony Diameter (mm)
50
45
40
35
30
25
20
15
10
5
c
e24
e48
e72
e96
e120
24
48
72
96
120
144
168
192
216
240
264
Time (hrs)

Figure 8. Growth curves for cultures of Microsporum canis exposed to 3-ATA oxygen for indicated intervals.

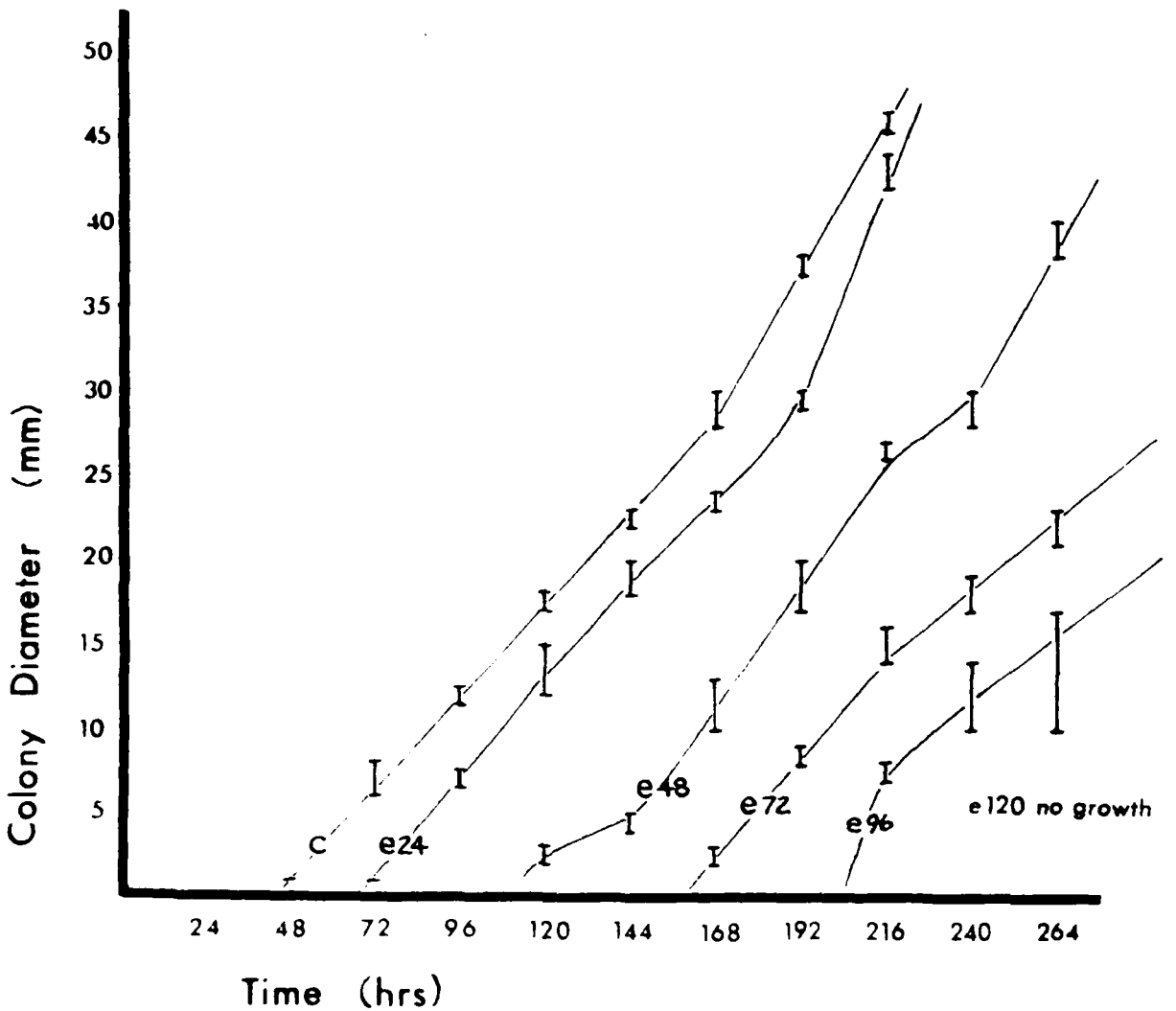

Colony Diameter (mm)
50
45
40
35
30
25
20
15
10
5
C
e24
e48
e72
e96
e120 no growth
24
48
72
96
120
144
168
192
216
240
264
Time (hrs)

Figure 9. Growth curves for cultures of Trichophyton tonsurans subjected to "treatment table" oxygen exposures.

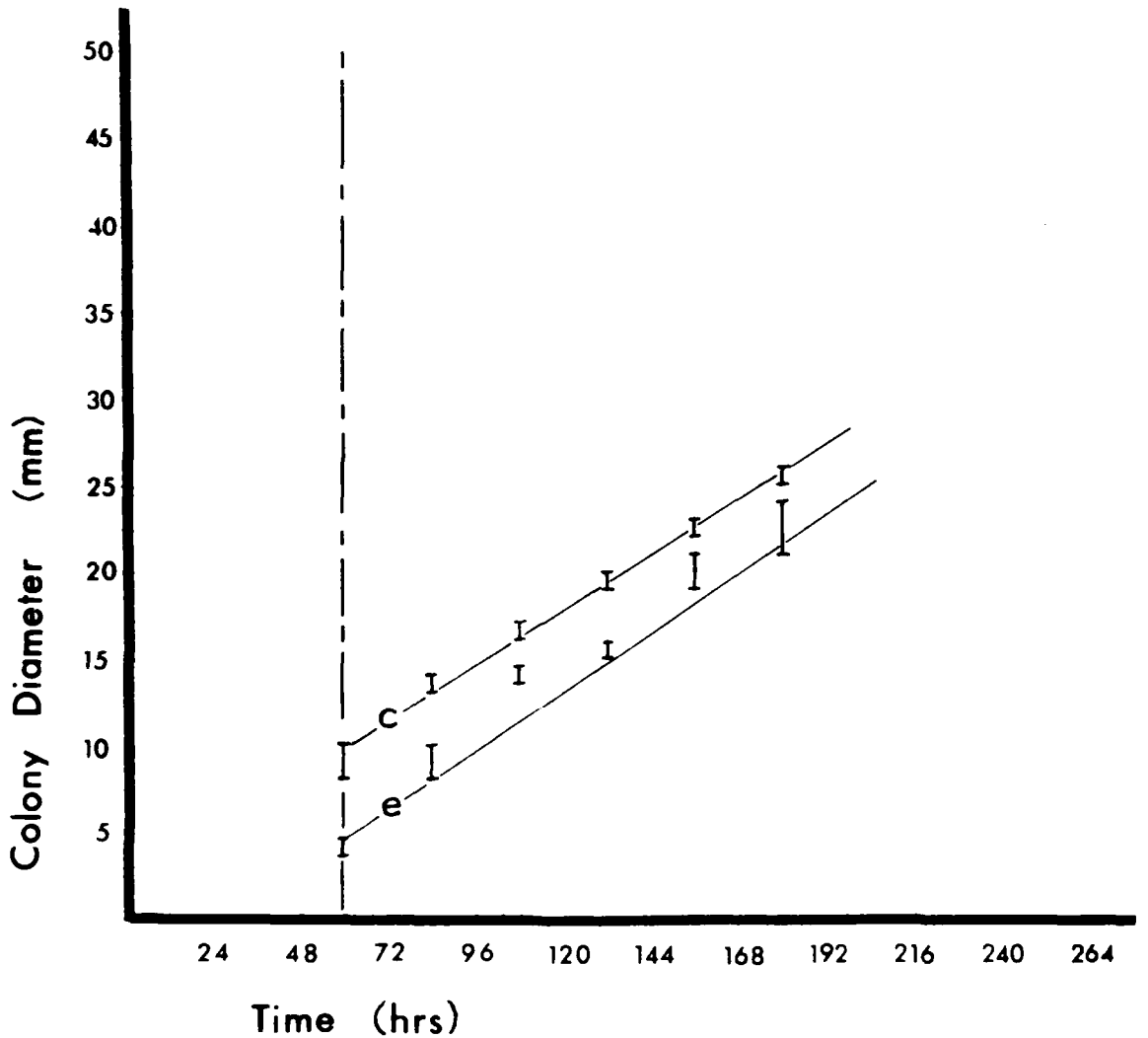

Colony Diameter (mm)
50
45
40
35
30
25
20
15
10
5
c
e
24
48
72
96
120
144
168
192
216
240
264
Time (hrs)

Figure 10. Growth curves for cultures of Trichophyton mentagrophytes subjected to "treatment table" oxygen exposures.

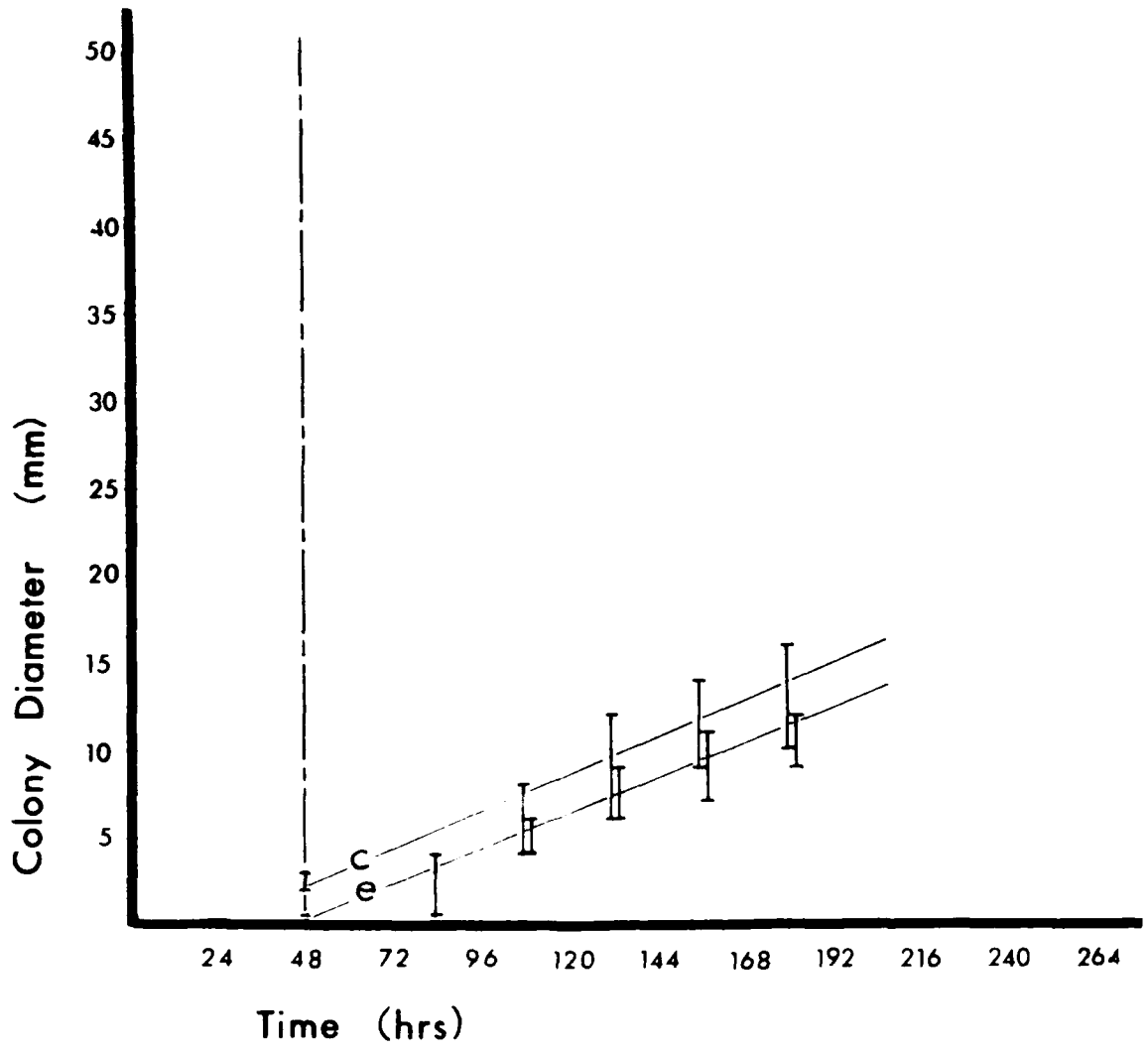

Colony Diameter (mm)
50
45
40
35
30
25
20
15
10
5
c
e
24
48
72
96
120
144
168
192
216
240
264
Time (hrs)

Figure 11. Growth curves for cultures of Microsporum gypseum subjected to "treatment table" oxygen exposures.

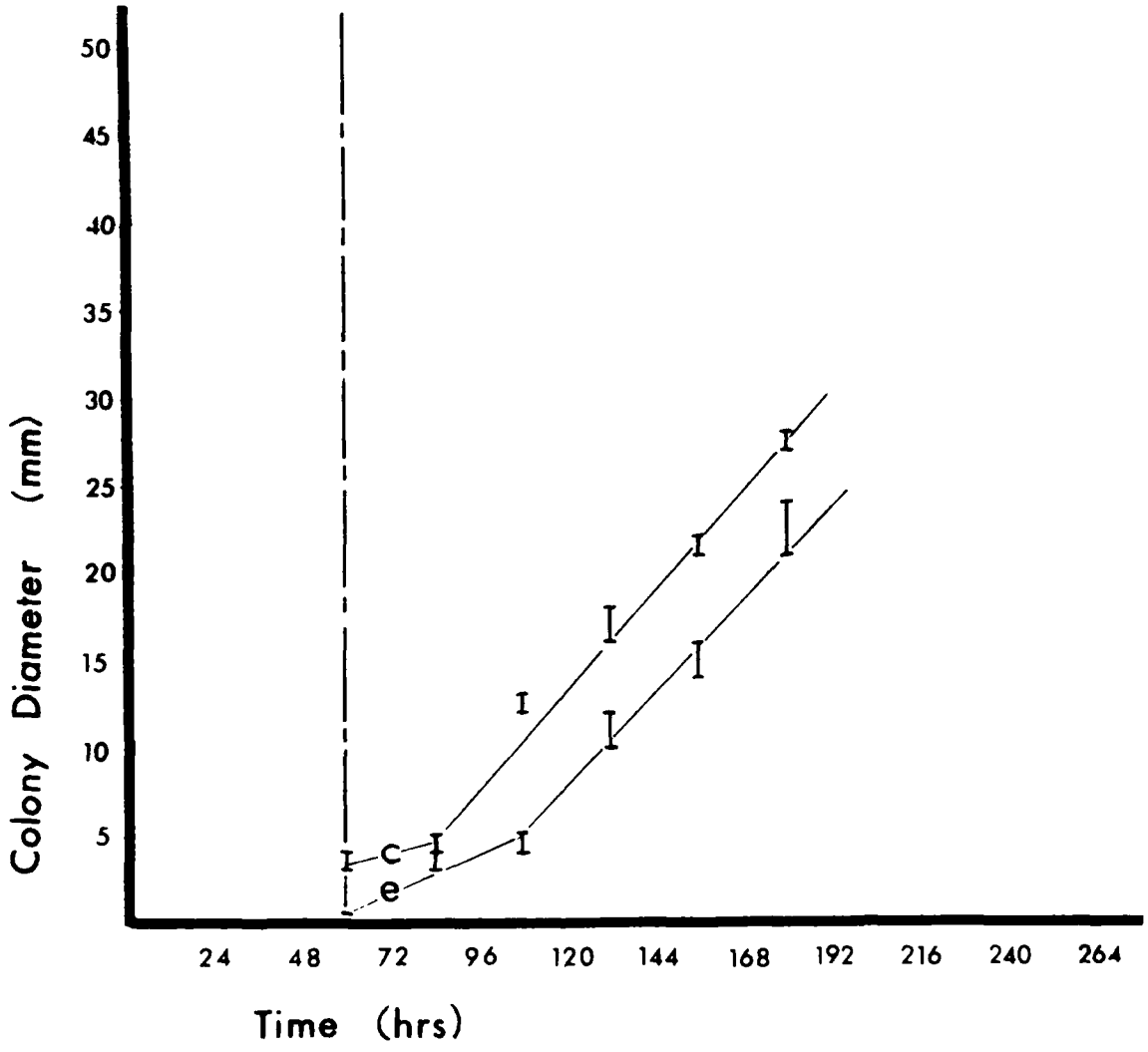
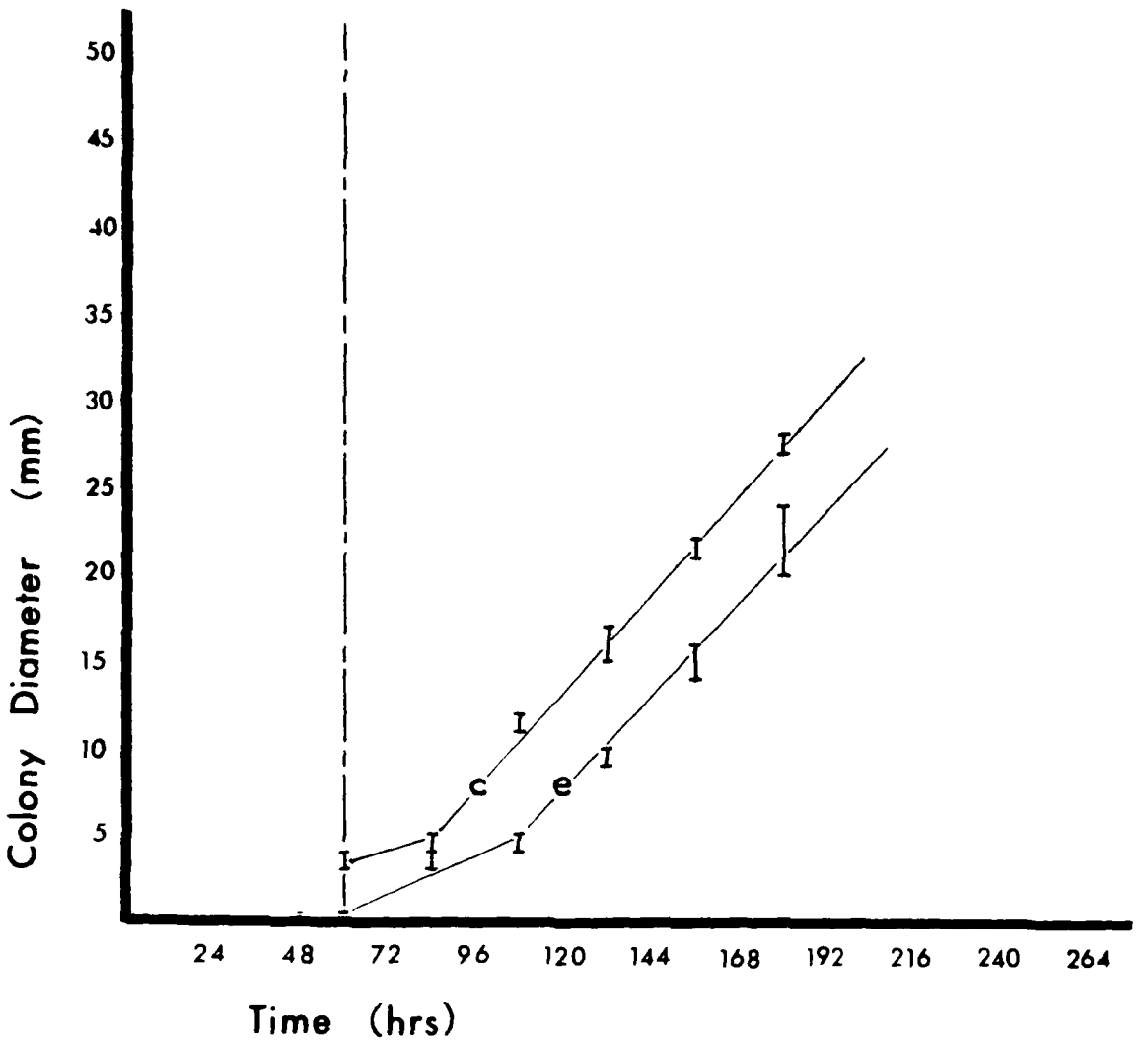

Colony Diameter (mm)
50
45
40
35
30
25
20
15
10
5
c
e
24
48
72
96
120
144
168
192
216
240
264
Time (hrs)

Figure 12. Growth curves for cultures of Microsporum canis subjected to "treatment table" oxvgen exposures.


Colony Diameter (mm)
50
45
40
35
30
25
20
15
10
5
c
e
24
48
72
96
120
144
168
192
216
240
264
Time (hrs)


FILMED
3-8