THE EFFECT OF A HOMOEOPATHIC COMPLEX (CANDIDUM, HELONIAS, MERCURIUS SOLUBILIS AND SEPIA OFFICINALIS) ON GROWTH AND GERM TUBE PRODUCTION OF *Candida albicans.*

ABSTRACT

The aim of the study was to establish the effect of a Helonias complex on the growth of Candida albicans in vitro in terms of growth rate, maximum specific growth rate, latent period before maximum specific growth rate and percentage germ tube production so as to establish the area of action of homoeopathic remedies.

The medicine consisted of Helonias, Sepia officinalis, Mercurius solubilis and Candidum a.a. in 20 % ethanol at 5, 7, 9, 12, 15, 30 CH potencies.

Candida albicans was cultured in human plasma inoculated from a stock culture of a standard optical density. The inoculated culture was then medicated and the growth monitored over 120 hrs. The experimental groups consisted of a control, an ethanol and one group for each of the various potencies used in the experiment. Each group contained triplicate samples and the experiment was conducted twice.

Growth was monitored by measuring optical transmittance of the samples from time 0 hrs to 120 hrs every 12 hrs. The results were recorded and analyzed to determine growth rate, maximum specific growth rate and latent period before maximum specific growth rate. Cell counts to determine percentage germ tube production were conducted at 0 and 120 hrs and the results recorded and analyzed.

Growth rate over 120 hrs showed no significant difference with only a 4 % standard deviation between the means of experimental groups. At 24 hrs significant difference was seen in the 7, 9 and 12 CH groups showed a 48 % reduction and 32 and 21 % increase in growth rate respectively.

Only 7 CH showed a significant increase in maximum specific growth rate with a 8 % standard deviation between the means of experimental groups.

The potencies 9 and 12 CH showed a significant (45 %) reduction in latent period before maximum specific growth rate and 7 CH showed a slight increase in latent period before maximum specific growth rate from the control from 22.29 hrs to 24 hrs.

Although cell counts were conducted, percentage germ tube production did not yield any credible results due to very high standard deviation within the experimental groups.

Although the 7, 9 and 12 CH groups showed a difference from the control in terms of growth rate and latent period before maximum specific growth rate the results will not be considered significant due to the time frame between the measuring of the samples i.e., 12 hrs. Further research is therefore recommended now required to establish the effect in the initial 24 hrs after exposure to the medicine and the effect of repeated medication over a long time frame eg. 120 hrs.

A dissertation in partial compliance with the Master's Diploma in Technology in the Department of Homoeopathy at Technikon Natal

Date of Submission: September 1995

I, Allen Peckham, do hereby declare that in respect of the following dissertation/thesis, " THE EFFECT OF A HOMOEOPATHIC COMPLEX (CANDIDUM, HELONIAS, MERCURIUS SOLUBILIS AND SEPIA OFFICINALIS) ON GROWTH AND GERM TUBE PRODUCTION OF Candida albicans."; as far as I know and ascertain no other dissertation/thesis exists, and all references detailed in the dissertation are complete in terms of all personal communication engaged in and published works consulted.

_____ 18/1/96

SIGNATURE OF CANDIDATE DATE

APPROVED FOR FINAL SUBMISSION

18 Jan. '96

Dr. H. Kasan BSc(Hons), MSc, GDE, PhD, MDP DATE

SUPERVISOR

THIS DISSERTATION IS DEDICATED TO

ALL THOSE WHO HAVE SUPPORTED ME

THROUGH THIS STUDY

AND PETER AND NOLA FRAZER

FOR ALL THEY HAVE TAUGHT ME

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My family and friends

LIST OF ABBREVIATIONS

a.a.	- equal quantities
AVE	- average
CH	- centesimal Hahnemanian
GR	- growth rate
LP	- latent period before maximum specific growth rate
MSGR	- maximum specific growth rate
OD	- optical density

TABLE OF CONTENTS

CHAPTER 3

CHAPTER 4

LIST OF TABLES

TABLE NUMBER **PAGE NUMBER**

LIST OF FIGURES

FIGURE NUMBER **PAGE NUMBER**

CHAPTER ONE

1.0. INTRODUCTION

1.1. PROBLEM STATEMENT

The purpose of this study was to establish the effect of a Homoeopathic complex containing Helonias, Sepia officinalis, Mercurius solubilis and Candidum on Candida albicans grown in plasma by examining growth and formation of germ tubes.

1.2. SUB-PROBLEMS

1.2.1. The first sub-problem was to establish the effect of a Homoeopathic complex containing Helonias, Sepia officinalis, Mercurius solubilis and Candidum on Candida albicans grown in plasma in terms of growth rate, maximum specific growth rate and latent period before maximum specific growth rate was reached.

1.2.2. The second sub-problem was to establish the effect of a Homoeopathic complex containing Helonias, Sepia officinalis, Mercurius solubilis and Candidum on Candida albicans grown in plasma in terms of the formation of germ tubes.

1.3. HYPOTHESIS

1.3.1. The first hypothesis was that the effect of a Homoeopathic Helonias, Sepia officinalis, Mercurius solubilis and Candidum on

Candida albicans grown in plasma would cause decreased growth rate, decreased maximum specific growth rate and an increased latent period before maximum specific growth rate is achieved.

1.3.2. The second hypothesis was that the effect of a Homoeopathic Helonias, Sepia officinalis, Mercurius solubilis and Candidum on Candida albicans grown in plasma would cause a decrease in the number of cells forming germ tubes.

1.4. DELIMITATIONS

1.4.1. Only one strain of Candida albicans was used in the experiment.

1.4.2. The remedies used in the experiment were in complex form.

1.4.3. The spectrophotometer had up to a 3 % variability in readings due to varying thickness of the walls of test tubes

1.4.4. Only cells with germ tubes with lateral or dorsal orientation can be counted under a light microscope.

1.5. ASSUMPTIONS

1.5.1. The spectophptometer provides an accurate measurement of cellular growth in the broth.

1.5.2. The medicine was prepared to manufacturer's specifications.

1.5.3. The broth was prepared to the manufacturer's specifications.

1.5.4. The law of "Similia Similibus Curentur". (Hahnemann, Organon) is valid.

1.6. DEFINITIONS

Oxoid Broth - a liquid medium in which Candida albicans was cultured.

Candida albicans - a singular cellular yeast like fungus.

Complex remedy - A homoeopathic medicine containing more than one medicine.

In Vivo - Experiments done on animals and humans.

In Vitro - Experiments done in the laboratory.

Sabouraud liquid medium. code CM147 :

- a broth for growing yeast and fungi. Produced by Oxoid marketed by Unipath LTD. Basingstake, Hampshire, England.

Spectrophotometer - an instrument used to measure the

transmission of light through liquid media ie., their optical density.

1.7. IMPORTANCE OF THE STUDY

Candidal infections are commonly treated homoeopathically and show rapid response, especially vaginal candidiasis (Boericke ,1991). Treatment of candidiasis occurs in three forms; 1 Symptomatic or functional remedies repeated often in cases of an acute attack(Used in low potencies (5 to 9 CH)); 2 Constitutional remedies, in cases where the patient has a history of repeated attacks of candidiasis, these are used in higher potencies 15 to 30 CH); 3 nosodal treatment using the infectious organism prepared homoeopathically to treat the infection (using a homoeopathic medicine prepared from the Candida albicans, Candidum to treat candidal infections) (Jouanny, 1991). In this study an attempt was made to establish the area of action of homoeopathic remedies in effecting a response in a Candida albicans infection.

This study was formulated to determine :-

(a) whether the Helonias complex had an effect on Candida albicans in vitro by combining all 3 forms of homoeopathic treatment,

(b) and to provide motivation for further research as to where and how homoeopathic medicines effect a response in vivo in the treatment of disease.

If there is inhibition of cell growth in the laboratory, then it may be assumed that the medicine used had an effect on the cells. If there is no inhibition of cell growth, then one may assume

that :-

(i) factors other than the medicine used influenced the bodies reaction and/or

(ii) the body's utilisation of the medicine used determined its reaction to the presence of Candida albicans during an infection.

The literature which was reviewed provided no conclusive results as to the effect of homoeopathic medicine on organisms grown in vitro but rather only stating whether there had been an overall inhibition of cell growth, but not how the growth was affected. Thus the way in which homoeopathic medicine effects a response in the treatment of a disease has not yet been determined and this study, if successful, could provide such information, thereby making homoeopathy more acceptable to both allopathic practitioners and laymen alike. This would further the acknowledgement of homoeopathy. In addition to this the results should establish whether Candida albicans may be treated in vitro with this complex.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

Candida albicans is described as a yeast like fungus. (O'Leary' 1989, Kockova, 1990, Barnett, 1990) despite it's characteristic of producing pseudohyphea (Kockova, 1990, Barnett, 1990), and chlamydospores with thick cell walls (Kockova, 1990), when seen on rice agar after one day's incubation (Kockova, 1990, Barnett, 1990). Candida albicans has been reclassified to the phylum Ascomycete and named Pichia (O'Leary, 1989, Kockova, 1990).

2.2 Medical importance

Candida albicans has been described as the most medically important yeast (Edwards, 1991) because of the role it plays in the normal microflora of human beings (Kockova, 1990), and since it causes the majority of yeast infections in human beings.

Candida albicans often grows on mucus membranes of the mouth, intestinal tract, genitourinary tract (O'Leary, 1989, Kockova, 1990, Edwards, 1991) and skin (O'Leary, 1989, Kockova, 1990, Edwards 1991). As part of the normal human flora it usually produces no symptoms or signs, in healthy individuals.

It is symbiotic and an opportunistic parasite (Ogilvie, 1987,

Kockova, 1990, Edwards, 1991). Infections are usually as a result of opportunistic over-growth in patients with compromised immunity (as in patients with other infections), those taking NSAID (non steroidal anti inflammatory drugs) (Edwards, 1991), those taking antibiotics, or those in which other factors suppress the competing micro flora are found (Barnett, 1990, Kockova, 1990, Edwards, 1991).

On the skin, Candida albicans produces a rash usually in warm moist areas eg: armpits, skin folds (eg: under large breasts, and between buttocks), and in areas covered by infants nappies, (nappy rash). In the oral cavity it causes oral thrush (Ogilvie, 1987, Kockova, 1990, Edwards, 1991) and other intestinal symptoms and signs (Edwards, 1991). In males it causes non gonococcal urethritis. In women, it is the most common cause of vaginal infection (Ogilvie, 1987, Barnett, 1990, Kockova, 1990, Edwards, 1991), 75% of women experience at least one episode in their life time (Edwards, 1991). The most common sign is a creamy white leucorrhea. Occasionally deep infection of individual viscera occurs. The lesions of vulva vaginal candidiasis resemble those of thrush, but produce more irritation, severe vulvar itching and a thick yellow cheesy discharge (Ogilvie, 1987, Barnett, 1990, Kockova, 1990, Edwards, 1991). Predisposing conditions, include the use of oral contraceptives, pregnancy, and diabetes mellitus. These cause an increase in glycogen and glucose in the vagina which stimulates growth of Candida albicans (Barnett, 1990) . The mode of transmission is by direct contact with the skin especially sexual contact. (Ogilvie, 1987, Kockova, 1990,

Edwards, 1991)

2.3 Homeopathic Treatment

The treatment of Candidiasis homeopathically, assumes 3 forms of treatment symptomatic, constitutional and nosodal (Boerika, 1991).

The nosodal treatment of Candidiasis, is homeopathically prepared Candida albicans called Candidum (Beoricke, 1991). Symptomatic remedies which are often used to treat acute attacks of candidiasis are Helonias and Mercurius solubilis (Jouanny, 1984). Constitutional remedies are used to treat patients with chronic repeated attacks of candidiasis, by stimulating the patient's immune system and by improving the patient's general wellbeing thereby raising the patient's resistance to infection, thus preventing further infection (Jouanny, 1984). Sepia officinalis is a commonly used constitutional remedy (Jouanny, 1984),and is prescribed according to its pathological symptom picture (Beoricke, 1991).

2.4 Homoeopathic research

At present it is not known how homoeopathic remedies actually work or where they work. Yet their effect on the body, as well as on groups of cells and specific organs is well recorded. The following experiments are examples of this.

The results of an experiment done by Koopman et al, 1990 investigating the inhibitory effect of Viscum album on the proliferation of human fibroblast cell lines, mouse tumour cells and human carcinoma cell lines, stated that there was no cytostatic evidence that tumour or malignant cell lines are killed, in vivo specific anti-tumour effects of the remedy have been described, but in vivo effects may have been brought about by a mechanism differing from in vitro activity for instance by stimulation of the immune system (Koopman et al., 1990).

A study conducted on juvenile frogs at the two-legged stage, in which Homoeopathic Thyroxine was given to them to see it's effect on their metabolism through their jumping activity, was found to markedly reduce their activity (Endler, 1991). This is contrary to the pharmacological action of thyroxine, but may be expected as a homoeopathic reaction, in light of the law of "Similia Similibus Curentur" (Hahnemann, Organon). This shows that homoeopathic medicines have an effect on the entire body it is given to (Endler, 1991).

During the present study if it is found that homeopathic remedies have a direct effect on the yeast cells then it may be assumed that the homoeopathic method of inhibiting cell growth and multiplication in Candida albicans infections is by directly acting on the micro-organism. If this is not found to be the case, then it may be concluded that the area of action lies elsewhere (Koopman, 1990).

2.5 VIRULENCE

A number of attributes which promote successful colonisation and invasion of tissues have been described (Mishra, 1992). Adherence of Candida albicans to a host surface is the first step of the infection process (Douglas, 1987) and subsequently Candida albicans secretes hydrolytic enzymes viz., proteinases and phospholipases which help in tissue invasion (Odds, 1988). In addition, morphological transition from yeast to mycelial form provides the organism with the means to propagate deeper into tissues (Odds, 1988). The recently observed phenomenon of high frequency switching has been implicated in providing resistance to antifungal agents (Soll, 1990). Antigenic variability provides an additional evasive advantage to this organism (Poulian, 1985). Thus a number of factors are cooperatively involved in the process of candidal virulence.

2.5.1 ADHESION

Adhesion of Candida albicans to the mucosal wall is of utmost importance for successful colonisation or the organism may be removed by the continuous bathing of body fluids over the mucosal membranes (Mishra, 1992). Studies conducted so far reveal a positive correlation between the ability of Candida species to cause infection and their ability to adhere to host tissues (McCourtie, 1984). Genetic studies have also suggested the involvement of adhesion in the virulence process. Mutants of Candida albicans with reduced ability to adhere exhibit an

attenuated virulence (Calderone 1985 ; Lehrer 1986).

2.5.2 MORPHOGENESIS

Like most pathogenic fungi Candida albicans exhibits dimorphism. The morphology of the fungus found in infected tissues is generally different from that of the propagule which infects (Mishra, 1992). Hence, morphological transition has been implicated in conferring survival advantage to the fungus (Shepherd, 1988).

Candida albicans exists in either yeast form or mycelial form depending on a variety of environmental factors (Odds, 1988).It is generally agreed that the hyphal form adheres better than the yeast blastospores to the hosts surfaces (Douglas, 1987, Odds, 1988). In addition hyphal tips have been seen as entities that penetrate the host tissues (Odds, 1988). These findings suggested involvement of morphogenesis in invading host tissues. However, a few reports have also indicated that blastospores can also be detected in infected tissues (Odds, 1988). So far these conflicting reports have prevented a general consensus on the role of morphogenesis in virulence.

The role of morphogenesis in virulence was supported by the isolation of another variant of Candida albicans unable to produce germ tubes which was less virulent than its parent (Buckely et al., 1986; Douglas, 1987). Interestingly, revision of its germ tube forming ability restored its virulence (Douglas

1987)

2.5.3 ANTIGENIC VARIABILITY

The antibodies against the cell wall components, viz., saccharide or peptide epitopes of mannoprotiens have convincingly demonstrated antigenic variations in the cell surface of Candida albicans (Mishra, 1992)

2.5.4 PHENOTYPIC SWITCHING

Most of Candida albicans strains are known to switch at high frequency between different phenotypes, that can be distinguished on the basis of their colonial and cellular morphology (Soll 1990). Three distinct switch systems namely , (i) the 3153 strain switch system, (ii) the WO-1 transition and (iii) the smooth white heavy myceliated transitional systems have been characterized. Among these switching systems white opaque transition has been studied the greatest. White and opaque cells differ in their ability to form hyphae (Soll, 1990). White cells form hyphae similar to other stains of Candida albicans,but opaque cells are unable to form hyphae under similar experimental regimen (Soll 1990). However, opaque cells once anchored showed hyphal formation (Soll 1990 ;Douglas 1987). Switching between phenotypes is thought to be accompanied by changes in adhesive properties and alterations in their wall ultrastructure and antigenicity.

Switching has been implicated to have modulatory effects on the pathogenic characteristics of Candida albicans by conferring plasticity to fungal cells, thereby avoiding the hosts immune system and developing anti-candidal drug resistance.

2.5.5 MOLECULES OF POTENTIAL IMPORTANCE IN VIRULENCE

Hydrolytic Enzymes:

Among various hydrolytic enzymes produced Candida albicans, protienases and phospholipases have been regarded as mayor attributes to its virulence. Other hydrolytic enzymes which are produced are acid phosphatase, autolysin, (1-3)-B-glucanase and chitobiase. Chitobiase(N-acetyl glucosaminidase) is known to be secreted during germ tube formation of Candida albicans (Shepherd, 1988). Its role in virulence has been suggested because a mutant of Candida albicans defective in chitobiase production was found to be less virulent than its parent (Shepherd, 1988)

Protiénases:

The presence of proteolytic activity in Candida albicans has been widely reported. Elevated levels of protienase activity in virulent strains of Candida albicans coupled with its higher antibody titre in candidiasis patients suggests its involvement in virulence (Mishra, 1992).

Phospholipases:

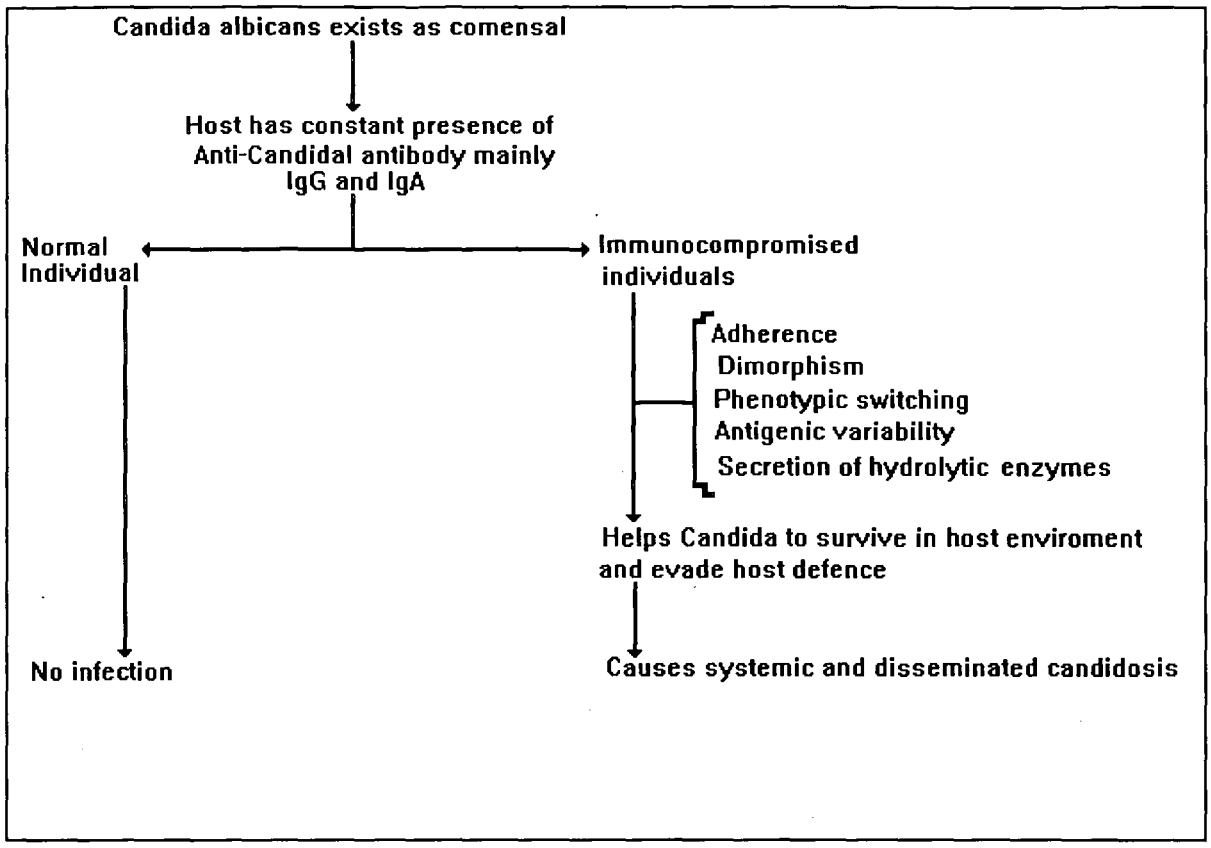
A correlation has been observed between phospholipase activity

and adherence of Candida albicans to human buccal epithelial cells (Soll, 1990). Pathogenic isolates of Candida albicans also show higher phospholipase activity and are able to adhere better to buccal epithelial cells than non-pathogenic yeasts (Mishra, 1992).

2.5.6 CONCLUDING REMARKS

Available evidence points to multiple factors which appear to work in concert for Candida to infect the host tissue. There is evidence to show that Candida albicans undergoes morphological changes, phenotypic switching and expresses variable antigens following adverse conditions encountered during environmental stress (Mishra, 1992).

From the information above it may concluded that there is no single mechanism which directly confers the virulence of the organism, but rather a multitude of different mechanisms which collectively and individually contribute to its virulence. So there is no single measure of virulence of Candida albicans.



A SCHEME FOR CANDIDAL VIRULENCE

A SCHEME FOR CANDIDAL VIRULENCE.

Although conflicting reports have prevented a general consensus as to the role of morphogenesis in virulence, it is generally agreed that the formation of germ tubes and the transition to a mycelial form contributes to adherence. In phenotypic switching with particular reference to opaque white phenotypic switching the form showing a greater ability to form germ tubes has a greater virulence. It is known that hydrolytic enzymes with reference to Chitobiase (N-acetylglucosaminidase) are secreted during germ tube formation (Sullivan, 1984). Genetic studies also show that variants of Candida albicans unable to produce germ

tube are less virulent than their parents. From the above text it can been seen that although there is not general consensus on the role of germ tube formation and that of the mycelial form in virulence, it is involved in phenotypic switching, adherence and with the secretion of hydrolytic enzymes. Due to this and the non availability of means to observe and measure the other factors which contribute to virulence, it was decided to measure the incidence of germ tube formation.

CHAPTER THREE

3.0 MATERIALS AND METHODS

The following was determined by preliminary laboratory work.

1. The primary stock preparation
2. The inoculation amount (1 ml / 100 ml).
3. The number of samples measured during the experiment using the spectrophotometer (11).
4. The time intervals for sampling (12 hrs).
5. The time over which the culture had to be grown to obtain a S - curve and approximately 30 % transmittance (120 hrs).

Note during these experiments :-

- Every time a bottle was opened or closed it was flamed.
- Whenever anything entered or was removed and returned to the laminar flow even for a few seconds it was sterilised with 70 % ethanol because this was the only way to avoid infection.
- Gloves where worn at all times because of the use of plasma.
- Whenever a pipetteman was used a new tip was applied.

3.1 Materials

Pipet man tips - 1 ml
- 5 ml
- 10 ml

Pipettes - 1 ml

Bottles - 15 ml
150 ml
200 ml
600 ml
1000 ml

Beakers - 1 L

measuring cylinders - 100 ml
- 200 ml

Test tubes - 15 ml
- 10 mm

Scissors

Scalpel

Bunsen burner

Lightmicro scope with minimum 400 objective, (Nikon Type 102).

Phase contrast microscope with camera 35 mm, minimum 400 objective

Spectrophotometer, (Milton Ray Spectronic 20 D).

Autoclave

Agitator

Automatic decanter

Incubator

Incubator room

Helonias complex 5, 7, 9, 12, 15, 30 CH - Helonias, Sepia officinalis, Mercurius solubilis and Candidum a.a. in 20 % ethanol; succussion 100 X per dilution. Prepared by Natura Homoeopathic Pharmacy in Pretoria, South Africa.

Computer SBM 386 SX 33, Lotus 123 version 5 for Windows

Fresh culture of C. albicans ATCC. 1028 obtained from National Institute of Medical Research.

3.2 Method of culture

3.2.1. Primary stock culture preparation

1. 2 Sabouraud Dextrose agar plates were inoculated with C. albicans and incubated for 48 hrs.

2. 6 * 100 ml of sterilised Sabouraud liquid medium prepared in

150 ml bottles

3. 2 Bottles of Sabouraud liquid medium were inoculated by means of a loop under sterile conditions in a lamina flow cabinet.

4. All 6 bottles were placed together in an incubator and incubated for 7 days.

5. When the stock was required, a blank was taken from one of the uninoculated bottles. Then inoculated broth was added to a uninoculated bottle until the correct optical transmission (40 %) was reached. A second uninoculated bottle was used and the procedure repeated with the second group in the experiment.

3.2.2. Plasma in the bottles.

1. 36 Units of fresh frozen plasma were obtained from the Natal Blood Bank.

2. These were stored in a deep freeze at - 6 C°

3. The equipment required in the experiment was then sterilised in a autoclave (120 C° for 20 min).

4. 12 Units were used per experiment. These were removed from the freezer and left out to defrost.

5. 10 Units of plasma and equipment were placed in a laminar flow

cabinet after it had been sterilised.

6. Using aseptic techniques, the plasma was transferred at 10 0 ml to as many 150 ml bottles as it could fill. These were then incubated till the next day to determine if any infection was present. Those without infection were then used in the experiment.

7. **Preparation of blanks :** Blanks were prepared so as to provide a standard against which all other cultures could be compared. 2 Units of plasma were transferred to a 600 ml bottle. 2 200 ml of plasma was transferred to 200 ml bottles. To these, 2 ml of sterilise distilled water and 1 ml of 20 % ethanol was added. This was in place of the culture and medicine. From these, 30 ml bottles were filled with 10 ml each (using a 10 ml pipetteman). 15 of these where placed in a fridge and stored at 5 C° to act as backup incase there was an infection in any of the other 15, which were then incubated, to determine if there was any infection. The uninfected samples were then used as blanks and were kept with the cultures at all times.

3.3. Inoculation of the plasma

1. Operating in a laminar flow and using aseptic techniques, 1 ml of stock culture and 0.5 ml of the appropriate medicine was added to each of the cultures (using a pipette man). The control received 0.5 ml distilled water and the ethanol group received 0.5 ml of 20 % ethanol instead of medicine.

The inoculated bottles were divided into 8 groups with 3 or 4 cultures in each group.

GROUP 1: CONTROL

GROUP 2: ETHANOL

GROUP 3: 5 CH

GROUP 4: 7 CH

GROUP 5: 9 CH

GROUP 6: 12 CH

GROUP 7: 15 CH

GROUP 8: 30 CH

3.4. Measuring the optical transmission

1. Immediately after inoculation and medication 5 ml from each sample was taken and the optical transmission measured, in glass cuvettes against a blank using a spectrophotometer (wavelength 615 Nm) . This was done to determine the original optical density at time 0.00 hrs so as to determine growth rates later.

2. Hereafter a 5 ml aliquot of each culture was measured every 12 hrs to determine the optical transition. This continued until 120 hrs.

3. The results were recorded on a table.

4. The results were then standardised and compared using a computer and presented in the form of tables (Table 1 and Figures

1 - 13)

3.5. Cell counts

1. Counting of cells and their morphology was undertaken by laboratory assistants at the Technikon Natal Microbiology Laboratory using light microscopes (100 and 400 objectives) and counting grids/chambers.

2. As samples were taken for measuring optical transmittance at time 0 hrs samples were also taken for counting.

3. At time 0 hours no dilution of the samples was necessary for counting, since the concentration of cells was very low.

4. At time 120 hours samples were again taken and counted. These were then diluted (10 exp-1) before being counted under the light microscope.

5. Results of both counts were then compared using a computer and presented in the form of a table to show percentage germ tube production (Table 1).

CHAPTER FOUR

4.0 RESULTS

4.1 Effect of the Helonias complex at varying potencies on the growth of *C. albicans*.

The results of experiments conducted in duplicate with triplicate or quadruplicate replicates each are presented in Figures 1 - 8. Composite data, namely, mean values of the replicates indicate that there is very little difference between the various experimental groups (Figure 9), There was only 4 % standard deviation in the average growth rate after 120 hours between the experimental groups (Table 1).

4.2 Comparison of growth parameters to determine the effect of the Helonias complex on the growth of *C. albicans*.

Computation of numerical values for average growth rate, average maximum specific growth rate and average latent period before maximum specific growth rate, was undertaken (Table 1).

The mean average growth rate per hour after 120 hours for each of the treatment groups indicated negligible difference between the means of the experimental groups i.e., a standard deviation of 0,03 per hour or 4 %.Standard deviation within the various treatment groups range from ± 0.01 i.e., 1.96 % (9 CH)to ± 0.07 i.e., 12.01 % (30 CH). The results are tabulated in Table 1 and

plotted as Figure 10.

The mean maximum specific growth rate for each of the treatment groups indicated a standard deviation between them of 0.18 per hour or 8 %. Standard deviation within the various treatment groups range from ± 0.05 i.e., 2.50 % (12 CH) to ± 0.41 i.e., 17.22 % (15 CH). The results are presented in Table 1 and plotted on Figure 11. The average standard deviation within the various treatment groups was 9 % (Table 1).

The mean average latent period before maximum specific growth rate of the treatment groups varied from 12 hours (9, 12 CH) to 24 hours (7 CH) with a standard deviation of 5 hours or 23 % between them. Standard deviation from the mean of each of the treatment groups varied from 0 hours i.e., 0 % (9, 12 CH) to 6 hours i.e., 34 % (5 CH). The results are shown in Table 1 and presented in Figure 12.

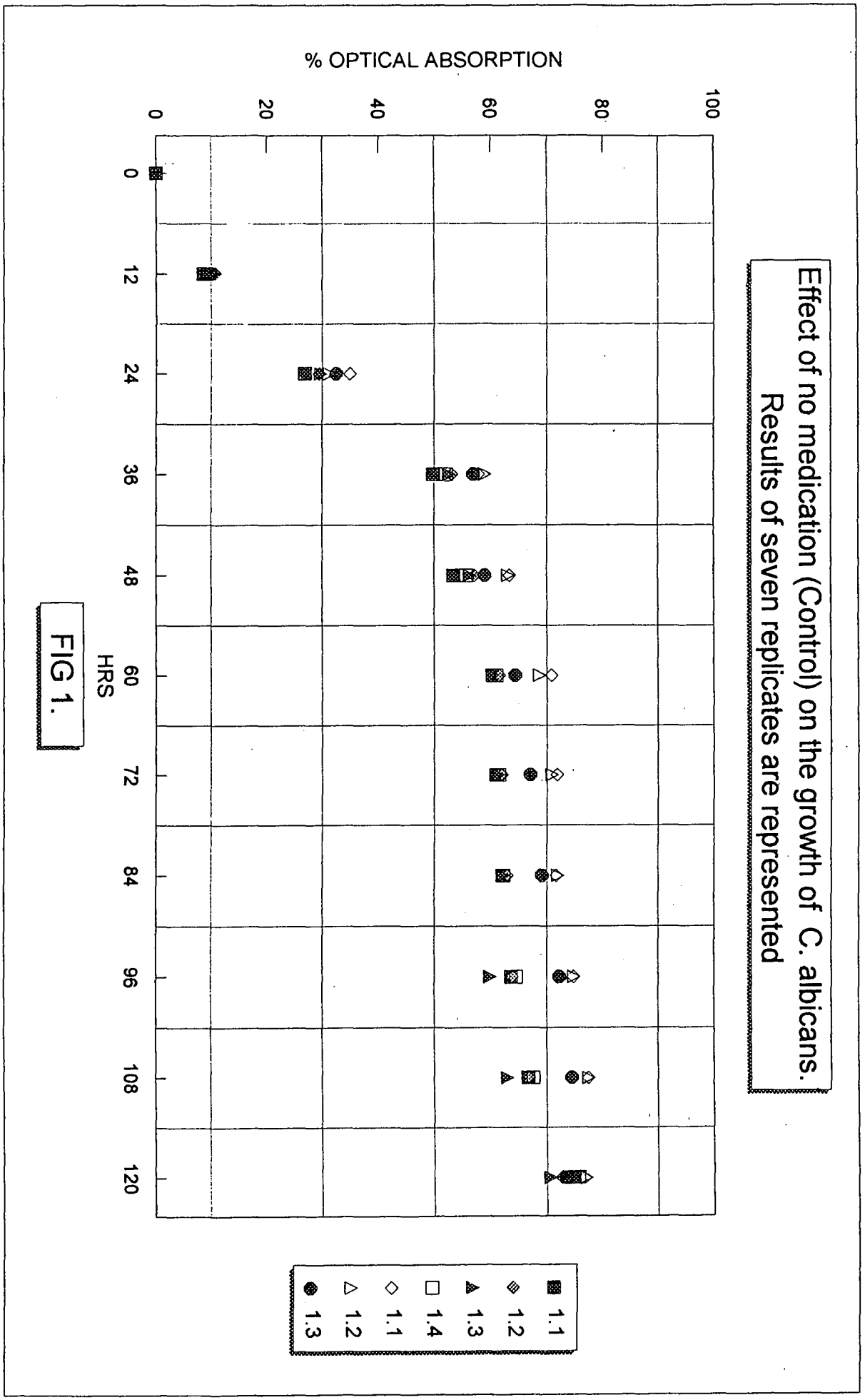

Effect of no medication (Control) on the growth of C. albicans.
Results of seven replicates are represented
% OPTICAL ABSORPTION
0
20
40
60
80
100
0
12
24
36
48
60
72
84
96
108
120
HRS
1.1
1.2
1.3
1.4
1.1
1.2
1.3

FIG 1.

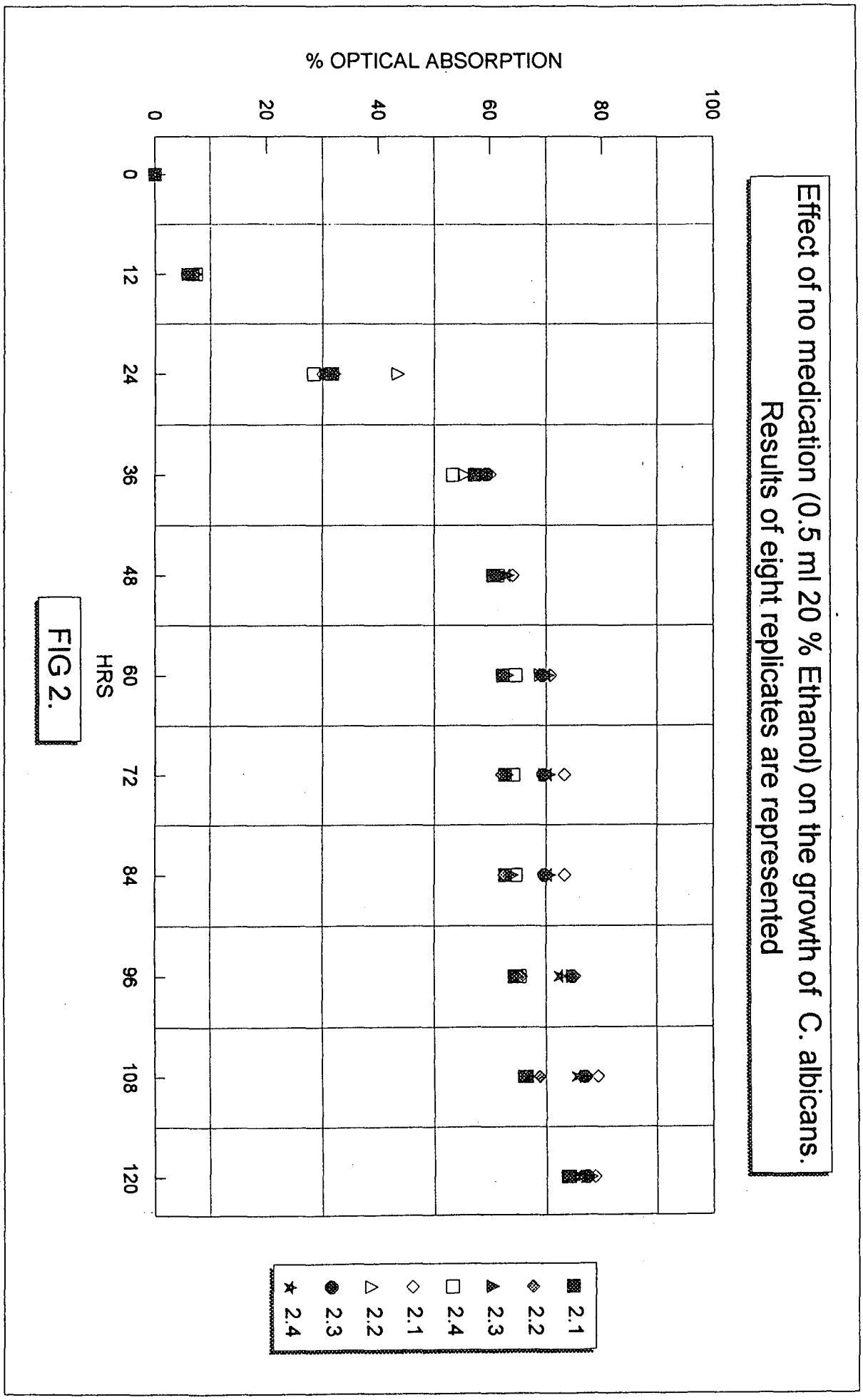

Effect of no medication (0.5 ml 20 % Ethanol) on the growth of C. albicans.
Results of eight replicates are represented
% OPTICAL ABSORPTION
0
20
40
60
80
100
0
12
24
36
48
60
72
84
96
108
120
HRS
2.1
2.2
2.3
2.4
2.1
2.2
2.3
2.4

FIG 2.

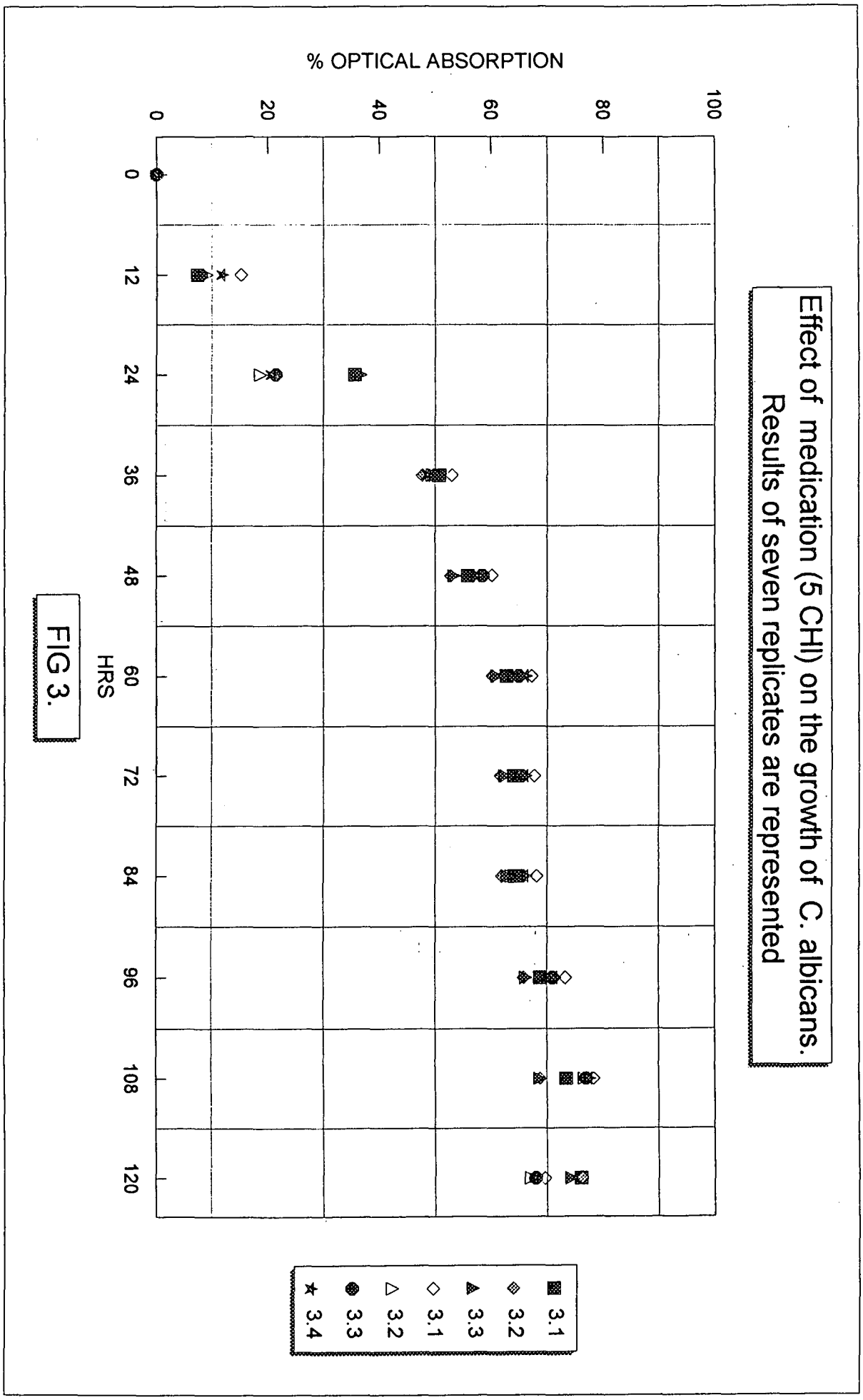

Effect of medication (5 CHl) on the growth of C. albicans.
Results of seven replicates are represented
% OPTICAL ABSORPTION
0
20
40
60
80
100
0
12
24
36
48
60
72
84
96
108
120
HRS
3.1
3.2
3.3
3.1
3.2
3.3
3.4

FIG 3.

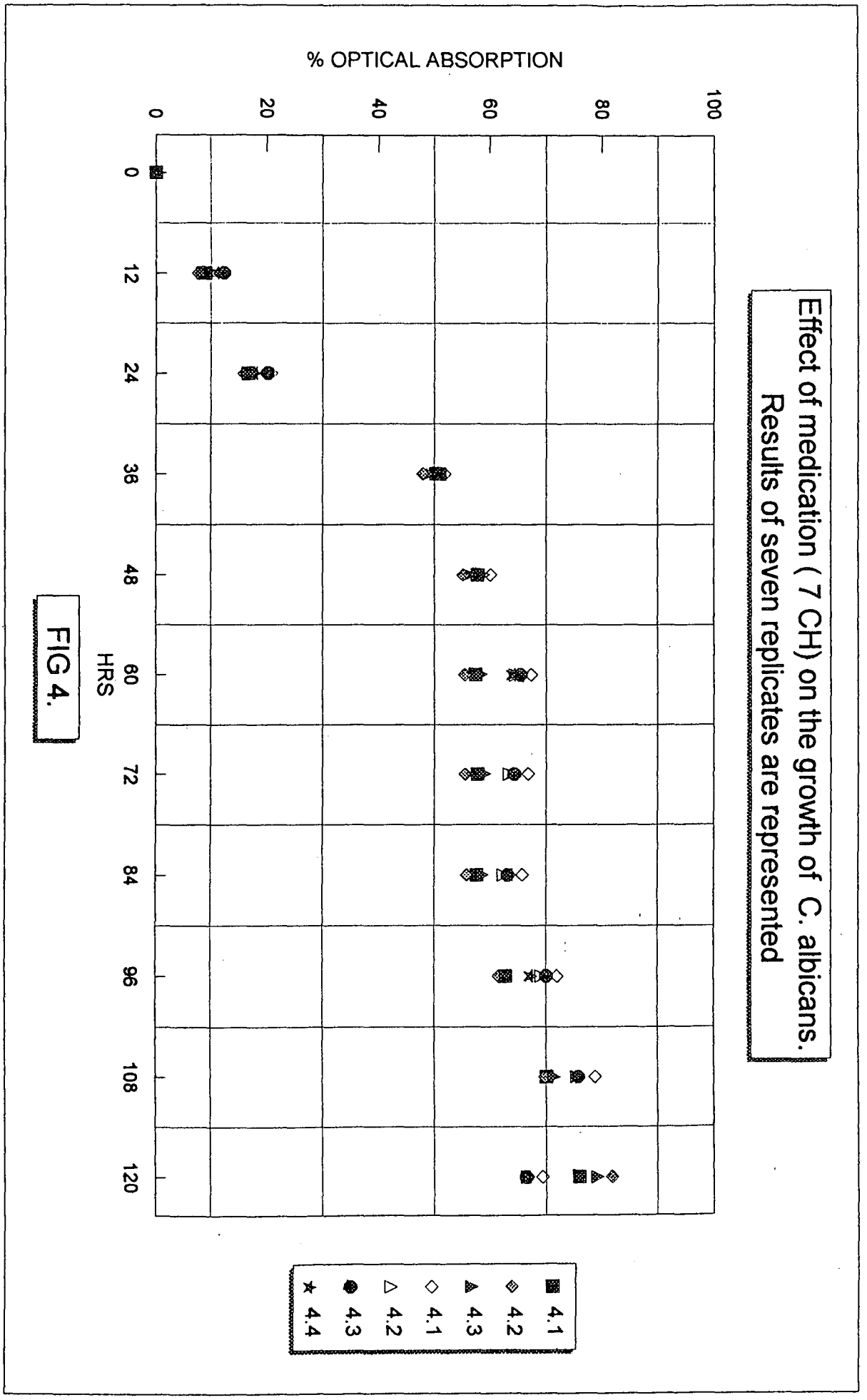

Effect of medication (7 CH) on the growth of C. albicans.
Results of seven replicates are represented
% OPTICAL ABSORPTION
0
20
40
60
80
100
0
12
24
36
48
60
72
84
96
108
120
HRS
4.1
4.2
4.3
4.1
4.2
4.3
4.4

FIG 4.

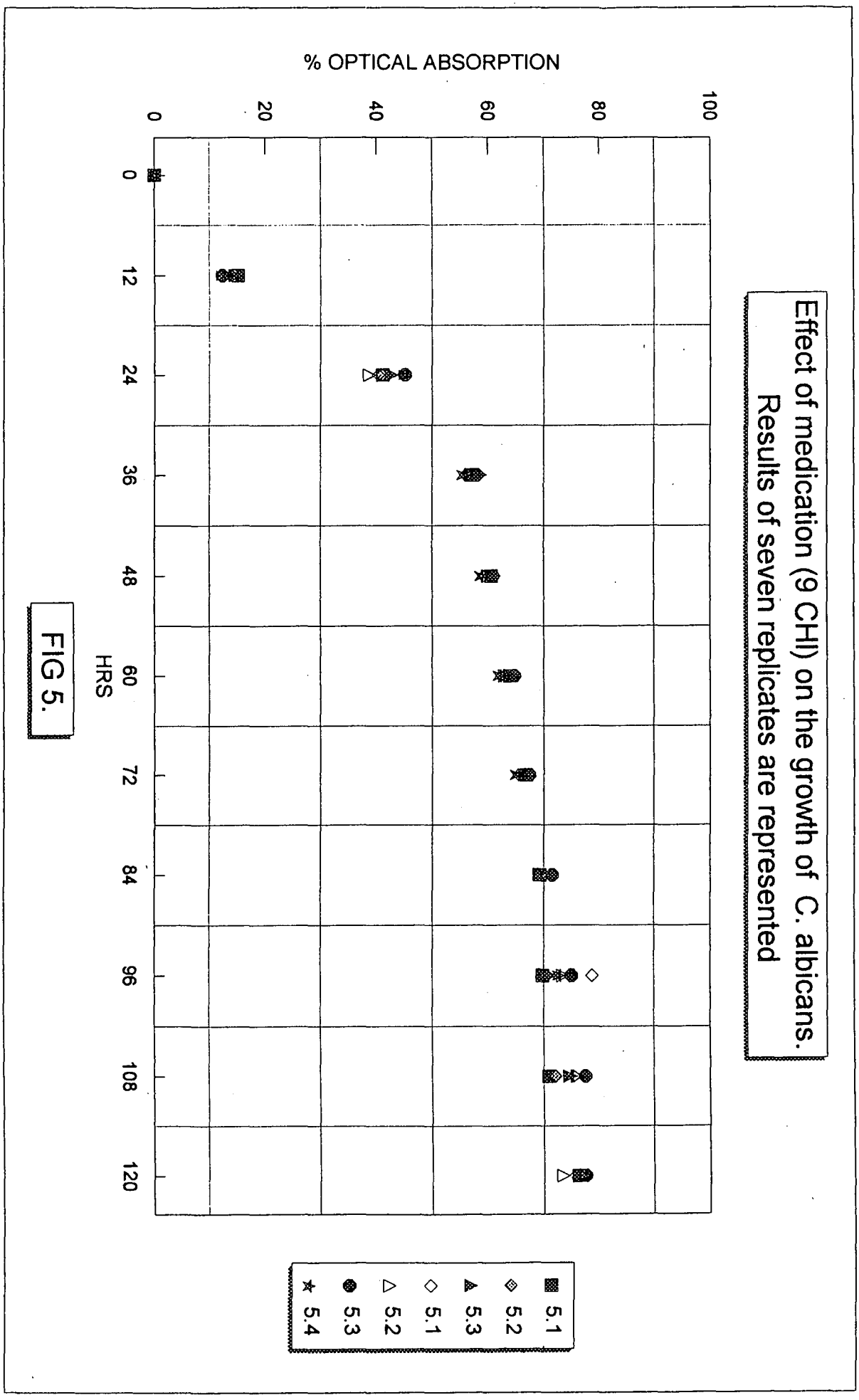

Effect of medication (9 CHI) on the growth of C. albicans.
Results of seven replicates are represented
% OPTICAL ABSORPTION
0
20
40
60
80
100
0
12
24
36
48
60
72
84
96
108
120
HRS
5.1
5.2
5.3
5.1
5.2
5.3
5.4

FIG 5.

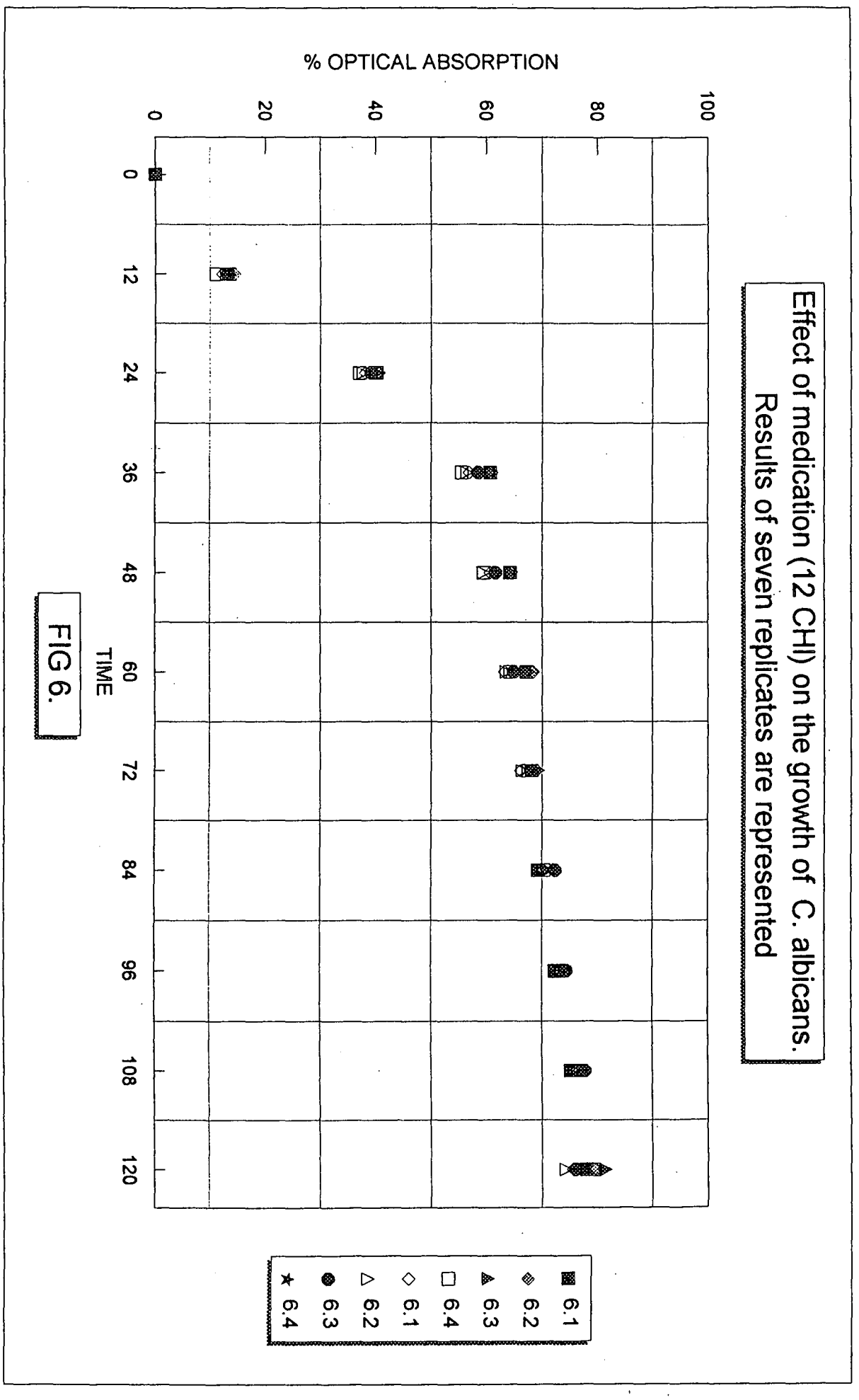

Effect of medication (12 CHl) on the growth of C. albicans.
Results of seven replicates are represented
% OPTICAL ABSORPTION
0
20
40
60
80
100
TIME
0
12
24
36
48
60
72
84
96
108
120
6.1
6.2
6.3
6.4
6.1
6.4
6.3
6.2
6.1
6.2
6.3
6.4

FIG 6.

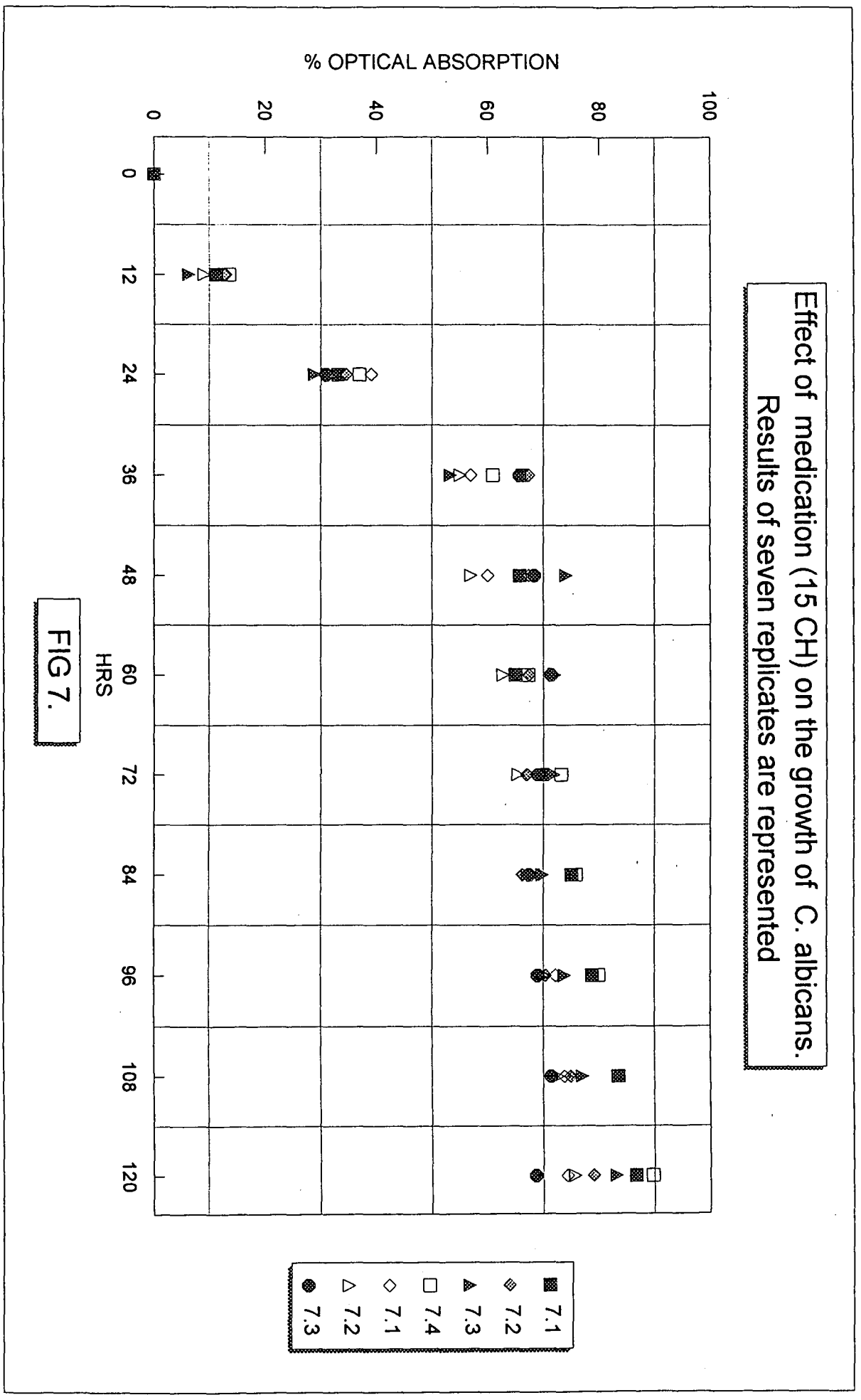

Effect of medication (15 CH) on the growth of C. albicans.
Results of seven replicates are represented
% OPTICAL ABSORPTION
0
20
40
60
80
100
0
12
24
36
48
60
72
84
96
108
120
HRS
7.1
7.2
7.3
7.4
7.1
7.2
7.3

FIG 7.

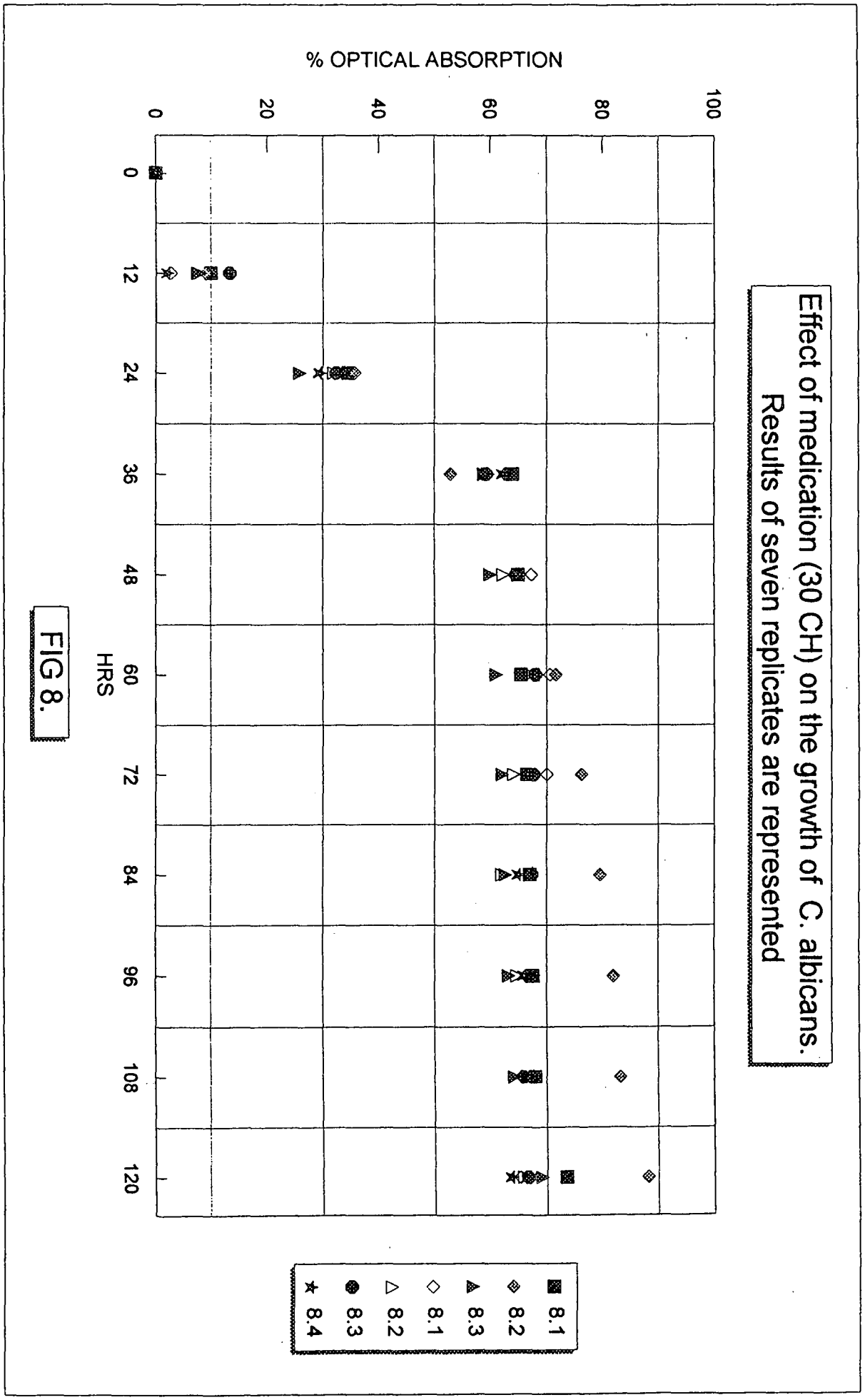

Effect of medication (30 CH) on the growth of C. albicans.
Results of seven replicates are represented
% OPTICAL ABSORPTION
0
20
40
60
80
100
0
12
24
36
48
60
72
84
96
108
120
HRS
8.1
8.2
8.3
8.1
8.2
8.3
8.4

FIG 8.

The effect of no medication (Control and 0.5 ml 20 % ethanol) and varying potencies 5; 7; 9; 12; 15; 30 CH) of the Helonias complex on the growth of C. albicans.

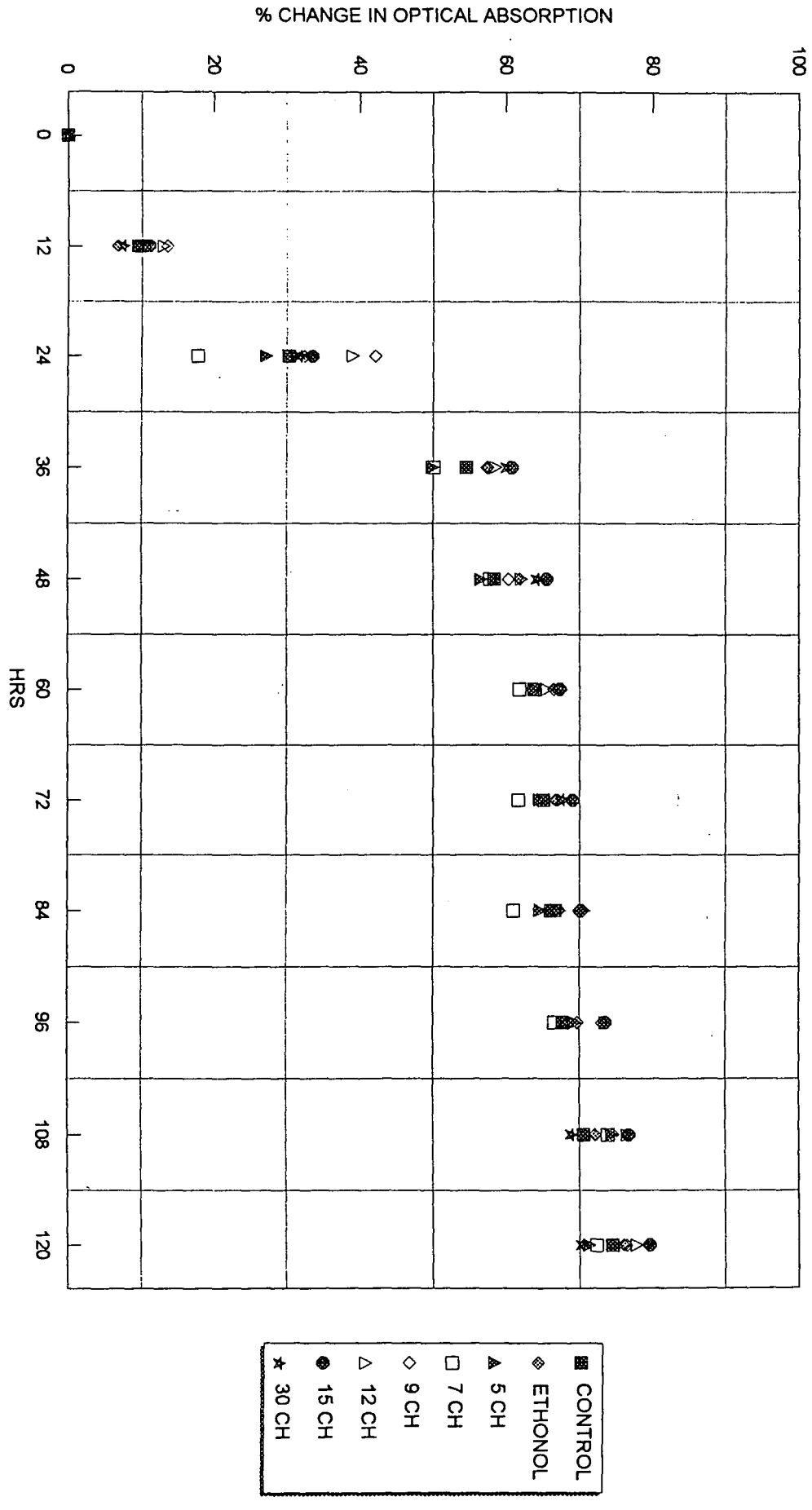


FIG 9.

TABLE 1.

Growth parameters of C. albicans in the presence and absence (Control; 0.5 ml 20 % Ethanol) of varying potencies (5; 7; 9; 12; 15; 30 CH) of the Helonias complex.

TREATMENT	GROWTH RATES PER HOUR			MAXIMUM SPECIFIC GROWTH RATES / HOUR			LATENT PERIOD BEFORE MAX. SPEC. GROWTH RATE		
	MEAN	SD	% SD	MEAN	SD	% SD	MEAN	SD	% SD
CONTROL	0.62	+ - 0.02	3.10	2.05	+ - 0.14	6.94	22.29	+ - 4.54	20.35
ETHONOL	0.63	+ - 0.02	2.50	2.36	+ - 0.32	13.47	22.50	+ - 4.24	18.86
5 CH	0.59	+ - 0.03	5.77	2.44	+ - 0.12	4.99	18.86	+ - 6.41	34.02
7 CH	0.60	+ - 0.05	9.06	2.70	+ - 0.14	5.06	24.00	+ - 0.00	0.00
9 CH	0.64	+ - 0.01	1.96	2.38	+ - 0.25	10.70	12.00	+ - 0.00	0.00
12 CH	0.65	+ - 0.02	3.33	2.16	+ - 0.05	2.50	12.00	+ - 0.00	0.00
15 CH	0.66	+ - 0.06	9.24	2.38	+ - 0.41	17.22	22.29	+ - 4.54	20.35
30 CH	0.59	+ - 0.07	12.01	2.48	+ - 0.23	9.20	22.29	+ - 4.54	20.35
AVERAGE	0.62		5.87	2.37		8.76	19.53		14.24
SD	0.03	% Diff	4.08	0.18	% Diff	7.74	4.55	% Diff	23.29

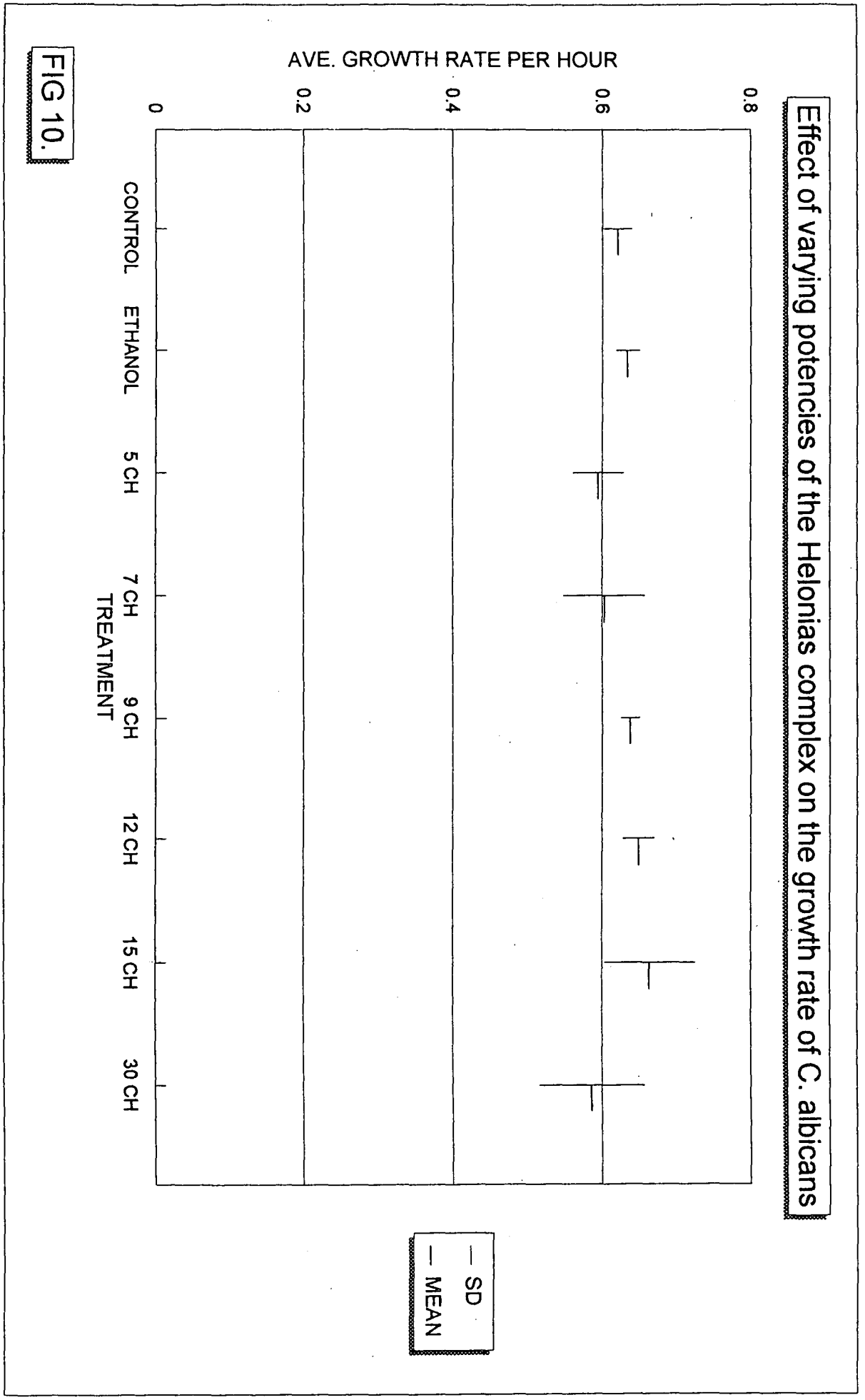

Effect of varying potencies of the Helonias complex on the growth rate of C. albicans
AVE. GROWTH RATE PER HOUR
0
0.2
0.4
0.6
0.8
CONTROL
ETHANOL
5 CH
7 CH
9 CH
12 CH
15 CH
30 CH
TREATMENT
SD
MEAN

FIG 10.

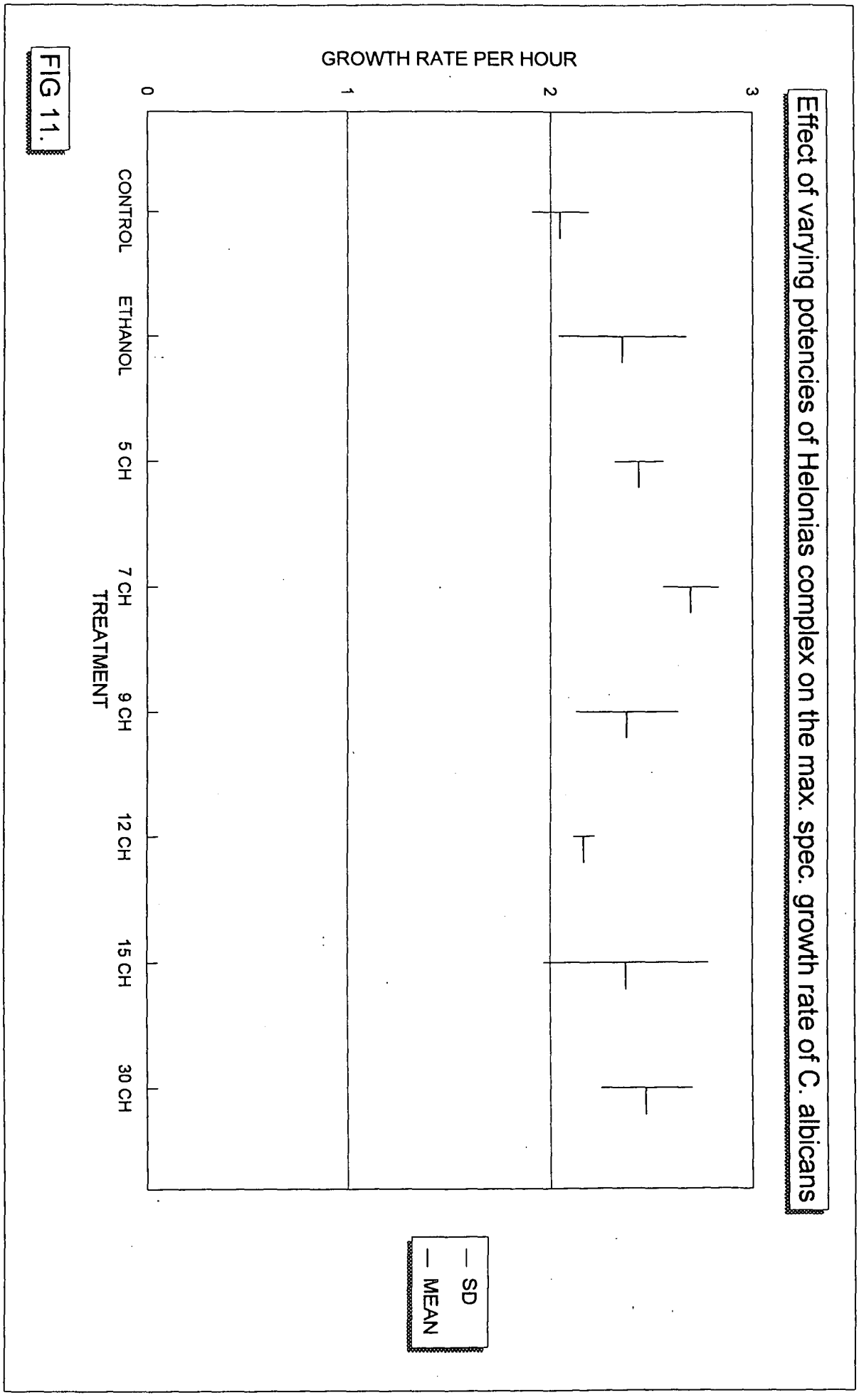

Effect of varying potencies of Helonias complex on the max. spec. growth rate of C. albicans
GROWTH RATE PER HOUR
0
1
2
3
CONTROL
ETHANOL
5 CH
7 CH
9 CH
12 CH
15 CH
30 CH
TREATMENT
SD
MEAN

FIG 11.

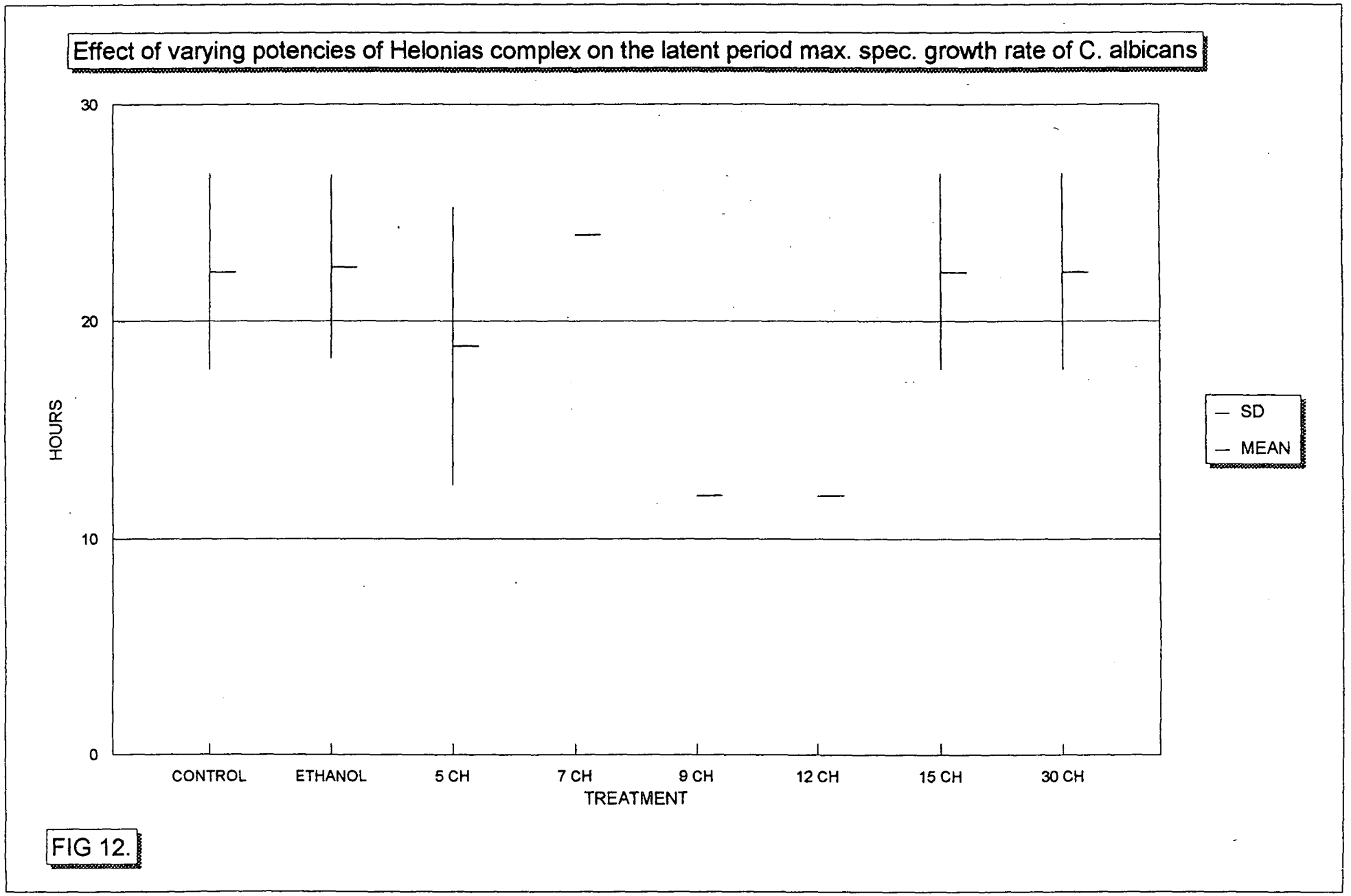

Effect of varying potencies of Helonias complex on the latent period max. spec. growth rate of C. albicans
30
20
10
0
HOURS
SD
MEAN
CONTROL
ETHANOL
5 CH
7 CH
9 CH
12 CH
15 CH
30 CH
TREATMENT

FIG 12.

4.3 Effect of the Helonias complex on the germ tube production of *C. albicans.*

The results of experiments conducted in duplicate with triplicate or quadruplicate replicates are represented by the means of the treatment groups in Table 2. The standard deviation between the means of the germ tube production of the various treatment groups after 120 hours is 8 ie 13 %. Standard deviation within the various treatment groups is indicated in Table 2, and range from ± 28 ie 42 % (Control)to ± 34 i.e., 80 % (12 CH).

TABLE. 2 The effect on the percentage germ tube production of C. albicans in the presence and absence (Control; 0.5 ml 20 % Ethanol) of varying potencies (5; 7; 9; 12; 15; 30 CH)of the Helonias complex.

GROUP	0 HRS AVE % G/T		SD	120 HRS AVE % G/T		SD	% SD
CONTROL	0.00	+ -	0.00	65.59	+ -	27.72	42.26
ETHONOL	0.96	+ -	2.72	64.38	+ -	29.24	45.42
5 CH	0.00	+ -	0.00	62.46	+ -	33.19	53.14
7 CH	0.00	+ -	0.00	59.95	+ -	40.72	67.91
9 CH	0.00	+ -	0.00	62.56	+ -	27.39	43.78
12 CH	0.00	+ -	0.00	42.94	+ -	34.24	79.75
15 CH	0.00	+ -	0.00	50.93	+ -	35.69	70.08
30 CH	0.00	+ -	0.00	57.68	+ -	36.45	63.20
SD			AVERAGE	58.31		AVERAGE	58.19
			SD	7.74	% DIFF	13.27	

* The reading at 0 Hrs is as a result of 1 cell with a germ tube and is considered insignificant.

CHAPTER FIVE

5.0 DISCUSSION

Using the Multiple Comparrison method and Least Significant Differences with a confidence level of 99 % the following was determined.

5.1 Effect of the Helonias complex at varying potencies on the growth of *C. albicans*.

The results as shown in chapter 4.1 (Figures 1 - 9) show no significant difference in the growth curves after 120 hours with only a 4 % standard deviation but there is a greater difference after 24 hours as can be seen on Fig 9.

5.2 Comparison of growth parameters to determine the effect of the Helonias complex on the growth of *C. albicans*.

5.2.1. GROWTH RATE

The mean average growth rate per hour after 120 hours for each of the treatment groups indicated little difference between the means of the experimental groups with a standard deviation of 0,03 per hour or 4 %.The average standard deviation within the various treatment groups was low (6 %). The results show a small difference in standard deviation of the means of experimental groups and indicate no significant difference within the groups

themselves.

The average growth rates at 24 hours vary considerably with a average mean growth rate per hour more than double that at 120 hours, 1.32 as compared to 0.62. There is also a marked difference between the various experimental groups taking the effect of ethanol into consideration. The mean growth rate per hour :-

GROUP	% DIFF FROM CONTROL	% SD WITHIN EXPERIMENTAL GROUP
CONTROL	0.00	9.60
5 CH	- 17.16	30.83
7 CH	- 48.32	10.71
9 CH	+ 31.92	5.75
12 CH	+ 21.83	3.56
15 CH	+ 3.58	10.69
30 CH	- 2.07	10.19

The above data shows that the 7 CH groups has a significantly lower average growth rate over 24 hours than the control. The potency 7 CH, showed a growth rate of nearly half that of the control. The 9 and 12 CH groups show a significantly higher average growth rate over 24 hours than the control group - the standard deviation within the groups is stated above.

The results after 120 hours show no significant variation from

Effect of no medication (Control and 0.5 ml 20 % ethanol) and varying potencies (5; 7; 9; 12; 15; 30 CH) of Helonias complex on the growth rate of C. albicans after 24 hrs.

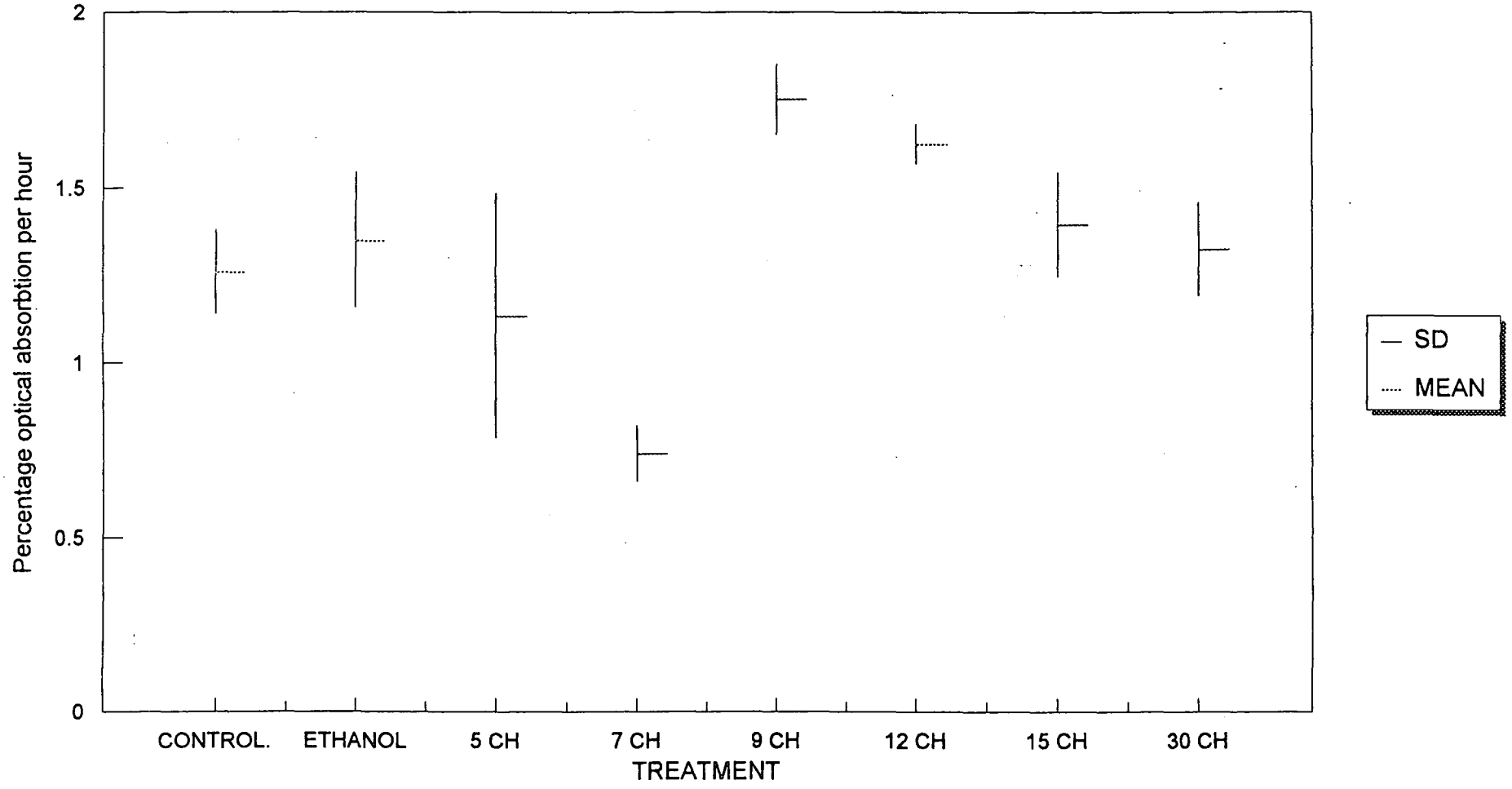


FIG 13

the control but at 24 hours there is a marked difference (Figure 13), 5 and 7 CH both support the hypothesis (1.3.1.) in that there is a decrease in the growth rate. 9 and 12 CH show an increase in growth rate :opposite to what was stated in the hypothesis. The decrease in growth rate may be attributed to a direct influence of the medicine on the cells. The increase in growth rate indicates that the medication in these potencies may have a direct effect on the cells stimulating growth. The explanation for cure lies in the homoeopathic law "Similia Similibus Curentur"; what cause a disease may also cure it.

The experimental groups were only medicated once at 0 hours. This explains why the difference is only visible at 24 hours, C albicans then proceeded to multiply as normal, as the effect of the medicine had diminished.

5.2.2. MAXIMUM SPECIFIC GROWTH RATE

The mean maximum specific growth rate for each of the treatment groups indicated a standard deviation between them of 0.18 per hour or 8 %. The mean maximum specific growth rates were all higher than the control (Figure 11) but if the effect of ethnol is taken into account only 7 Ch shows a significant difference, with a maximum specific growth rate of 17 % above the control after taking into account the effect of the ethanol. This may be attributed to the fact that it shows the lowest growth rate at 24 hours and the samples were only medicated once at 0.00 hours. The absence of any significant effect on the maximum specific

growth does not support the hypothesis (1.3.1.). The maximum specific growth of 7 CH which is greater than the control does not support the hypothesis, this may be due to the effect of the medicine diminishing after 24 hours and therefore allowing a period of growth greater than normal.

5.2.3. LATENT PERIOD BEFORE MAXIMUM SPECIFIC GROWTH RATE

The mean average latent period before maximum specific growth rate of the treatment groups varied widely from 12 hours (9, 12 CH) to 24 hours (7 CH) with a standard deviation of 4.55 hours or 23 % between them. 9 and 12 CH show a significant difference, 45 % shorter (taking into account the effect of ethanol) than the control, especially considering there is a 0.00 % standard deviation within the 9 and 12 CH experimental groups. Standard deviation from the mean of each of the treatment groups varied from 0.00 % (9, 12 CH) to 6.41 hours i.e., 34 % (5 CH). The results are indicated in Table 1 and Figure 12. The 7 CH group which showed the largest maximum specific growth rate showed the longest latent period before maximum specific growth rate 24 hours with 0.00 % standard deviation within the experimental group. These results, with little or no deviation suggest accuracy, but one must note that both high and low standard deviations are due to the fact that the readings for latent period before maximum specific growth rate could only be taken 12 hours apart because this was the time frame between readings, and any difference between results could have been nullified or accentuated by the time frame. As in a few cases only a few

percentage optical absorption points separated the selection of which the latent period would be recorded i.e., 12 hours or 24 hours (Appendix 1).

The increase in the latent period before maximum specific growth rate in the 7 CH control group supports the hypothesis (1.3.1.), but is not significant. There is a significant decrease in the latent period before maximum specific growth rate for the 9 and 12 CH groups which conflicts with the hypothesis (1.3.1).

5.3 Effect of the Helonias complex on the percentage germ tube production of *C. albicans* cells.

These results are not considered accurate with a standard deviation of 7.74 or a percentage standard deviation of 13 % from the average of the means of percentage germ tube production and a average percentage standard deviation of 58 % within the experimental groups. This is due to the large difference in the number of cells counted in each sample within the experimental groups.

There were numerous areas where the experiment could have been improved;

1. **The cell count** was of no benefit, standard deviation with in the experimental groups in terms of percentage germ tube production and cell numbers was just too high ie, 58 %. This was probably because there was only one count per sample and C.

albicans has a very sticky surface causing the cells to clump and not allow them to separate very readily even with shaking thus making dilutions within the cell counting process inaccurate.

2. **Spectrophotometry :** Areas where improvement could have been made:-

Plasma, the growth medium for this experiment, is not of uniform optical density so calculations had to be made to standardise the results allowing an element of error to be introduced.

The blanks against which the samples were measured were of constant optical density because they all came from the same units of plasma which were mixed together. Plasma, although an ideal growth medium to determine the effect of a medication on an organism in terms of it's application in humans, need not be used in further experiments as the cell counts of germ tube production have proved inaccurate, the original reason for its choice. This will allow the use of chemical growth media; increasing accuracy in terms of spectrophotometry because of their uniform optical density.

The use of disposable test tubes in the experiment increased accuracy because C. albicans sticks to glass and even during cleaning does not easily or completely come off and combined with soap residues create large inaccuracies. Inaccuracy occurred because there are tiny variations in the glass thickness usually causing a 0.2 but up to 3 % variation in the optical transmittance readings which was reduced by taking 3 separate

readings and recording the mean. This inaccuracy could have been overcome by the use of test tubes with a specific wall width but would become very expensive since the tubes can only be used accurately once.

3. There was only one strain of C. albicans tested during the experiment vitos ATCC 1028.

4. Culture of the organism :- Although the culture medium (plasma), the correct pH, agitation and the correct temperature was present, there is still room for improvement. Oxygen could have been pumped into the samples during incubation. However, the bottles were opened every 12 hours, pumping oxygen increases the risk of infection and due to financial constraints this could not be done and was not deemed essential.

5. The experiment could only be repeated twice due to the fact that the Natal Blood banks surplus plasma stock had been exhausted. Shortages were being experienced. Plasma could not be obtained elsewhere in sufficient quantities since all provinces were experiencing shortages. Due to this it was both impractical and unethical to continue the experiment.

CHAPTER SIX

6.0 CONCLUSIONS

6.1 Effect of the Helonias complex at varying potencies on the growth parameters of *C. albicans*.

As can be seen in the Results and Discussion there is no significant effect on growth rate at 120 hours for any of the experimental groups, yet at 24 hours there is a significant reduction in growth rate for (48 % reduction) the 7 CH group, at the same time there is a significant increase in growth rate of 32 % and 22 % for 9 and 12 CH respectively. 7 CH was the only group to show an significant increase (17 % between 24 and 36 hours) in maximum specific growth rate. The latent period before maximum specific growth rate was increased to 24 hours in the 7 CH experimental group and was significantly reduced to 12 hours for the 9 and 12 CH groups.

The experiment shows that in the 24 hours after medication the complex at 7 CH inhibits growth while at 9 and 12 CH significantly stimulate growth (Figures 9, 11 and 12).

6.2 Effect of the Helonias complex at varying potencies on percentage germ tube production of *C. albicans*.

The percentage germ tube production cannot be used to draw any conclusions since the standard deviation within the various

experimental groups is too great (58 %).

6.3 Summary

The results show a direct effect on the cells of C. albicans by the Helonias complex in 24 hours after initial exposure to the medicine. 7 CH shows decrease in growth rate and an increased latent period before maximum specific growth rate supporting the hypothesis 1.3.1., 9 and 12 CH show a increased growth rate and a decreased latent period before maximum specific growth rate showing the opposite of the hypothesis 1.3.1.

The experiment shows that certain potencies of the homoeopathic Helonias complex have a direct effect on the cells of C. albicans either stimulating or decreasing growth in the 24 hours after initial exposure. Further studies should be designed to examine the initial 24 hours after exposure to the medication and to determine the effect of repeated doses of the Helonias complex over a 120 hour period.

CHAPTER SEVEN

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