THE OHIO STATE UNIVERSITY

THE EFFECTS OF LOW MAGNETIC FIELDS ON THE GROWTH OF SELECTED MICROORGANISMS

A Thesis

Submitted in Partial Fulfillment of the Degree

Bachelor of Science

DEPARTMENT OF GEOLOGY & MINERALOGY

by

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INTRODUCTION

During the past several years, much discussion has centered around the possible relationship of polarity reversals in the earth's magnetic field and similarly timed extinctions of certain marine planktonic organisms. Results from marine paleomagnetic and biostratigraphic marine core samples point to such a correlation and it has been proposed that low geomagnetic fields, such as found during periods of reversals of the earth's polarity, are in some way responsible. Buffer (1963) and Simpson (1965) both proposed that increases in the cosmic ray flux at the earth's surface, as a result of the periods of low geomagnetic field intensity, could increase mutation rates enough to alter evolutionary trends significantly. However, such an increase in cosmic radiation would be small (7%) and the rays would be filtered out by the water before reaching marine organisms living more than a few centimeters below the surface.

The magnetic field of the earth is known to have reversed in polarity at least nine times in the last four million years (Harrison, 1968). In core samples taken by Hayes and others (1969) from the Pacific Ocean floor, several instances of faunal boundaries and reversals coincide within the sampling interval. Other core samples taken in Anarctica by Hayes and Opdyke (1967) also show that extinction of several species of foraminiferan were closely correlated with magnetic reversals. At least two of these extinctions could not be correlated with any climatic (temperature) changes as is often observed with such extinctions.

The research undertaken by this writer was a pilot attempt to find out if a low geomagnetic field in itself has a direct effect upon metabolism of certain microorganisms as opposed to the influence on metabolism over an extended period of time as proposed by Buffer and Simpson. Such an experiment requires the growth of microorganisms in an artificially produced, magnetic field free space, then the recording of any changes in growth rates or appearances as compared with that of the same species grown in the earth's normal magnetic field, with all organisms otherwise subject to the same environmental factors.

Ideally, in order to determine if such extinctions were directly related to the existence of a low geomagnetic field, one would want to study organisms of a similar nature to those that had become extinct under such conditions. However, because of limited funds and equipment, it was impossible to reproduce the sea water environment capable of supporting such organisms.

RESEARCH AND DATA

The nature of this experiment and the problems involved were discussed with Dr. Patrick Dugan of the the Ohio State University Department of Microbiology. He suggested that if the presence of a low geomagnetic field directly affected past faunal irregularities, then a laboratory environment providing a low ambient magnetic field would most likely have similar effects on bacteria. Thus the first phase of this experiment was organized to study the effects of a low ambient magnetic field on the growth rates of various bacteria.

Such a low ambient magnetic field was available in the paleomagnetic laboratory at the Ohio State Department of Geology and Mineralogy where a two meter Helmholtz core system is set up for thermal demagnetization studies in a zero ambient field (figure 1).

The initial step of this experiment was to construct shelves which could be placed within the Helmholtz system to hold the bacteria containers. Measurements were taken and a plan made for constructing shelves to fit around the furnace within the Helmholtz system. A three shelf platform was designed with the center shelf 12.75 inches high and level with the base of the furnace where the ambient field is nearest to zero. The shelves were cut and assembled according to plan using non-magnetic brass wood screws (figure 2). Then the shelves were carefully placed within the coil and glued to ensure stability. The construction was such that the shelves could be adjusted vertically if needed.

A potential problem existed from the excess heat created when the electric furnace was turned on for thermodemagnetization experiments. When operating, the 700°C internal temperature of the furnace causes a rise in the surrounding air temperature by as much as 10°F. In addition, when the furnace was shut down and the hood raised to cool the rock samples, the temperature in the shelves jumped an additional 20°F. A simple solution to this was to line the interior sides of the shelves with aluminum cooking foil (figure 3). This prevented the temperature on the shelves from rising more than 1°F during any phase of furnace operation.

Next, the intensity of the magnetic field was measured at various positions on the shelves with a portable Schonstedt milligauss meter. Each shelf was marked off with an identical grid system with lines drawn at 10 centimeter intervals. Measurements were taken at the intersections of the grid lines with the meter probe pointed north, south, east, and west. The vertical measurements were made only with the probe pointing down because the lead wire coming from the probe prevented accurate vertical "upward" measurements (figure 4). Because of this, the

vertical intensities were the least accurate since the north/south and east/west intensities were averaged. The intensities were converted to gammas and recorded for each shelf (figures 5,6,7).

PHASE I

As stated, the initial phase of the experiment involved growth of bacteria in petri dishes. Because of the writer's lack of training in microbiology, observation of changes in bacterial growth patterns had to be restricted to visual examination. In addition, learning the proper method for preparation of sterile petri dishes took considerable care and practice.

The growth medium selected for the bacteria was Tripricase soy agar, an all purpose medium that readily supports growth of the bacteria used in this experiment. The agar was prepared by mixing 40 grams of agar powder with one liter of distilled water in a flask, then slowly heating the solution to a boil (with constant agitation), and finally autoclaving the solution for 15 minutes at $200^{o}C$ for sterilization. Because the agar was extremely hot at this stage, extreme care was taken during handling. Next the agar was poured into petri dishes. The procedure was as follows: the stopper was removed from the flask and the rim passed through the flame of a Bunsen burner to insure sterility (a process called "flaming"). The agar was poured into three dishes at a time, the rim of the flask flamed and the stopper replaced. This process was repeated until the flask was empty, yielding 44 dishes.

Because the agar was hot when poured, steam quickly condensed on the insides of the petri dish lids. When the steam cooled and condensed, it would drip onto the agar, increasing the chances of contamination. Because of this, the lids were left slightly ajar until the steam escaped. However, leaving the lids off proved to be a mistake because 42 of the 44 dishes showed signs of contamination within two days. A second attempt yielded better results. When these dishes were poured, the lids were put on immediately and the dishes inverted after the agar hardened. This caused the condensed steam to remain in the lids and negated the requirement to leave the lids ajar. The result was that none of the new dishes showed signs of contamination after two days.

A dozen species of non-pathogenic bacteria were selected from a group currently in use by microbiology classes because all were readily available. These species would be grown in the petri dishes, watching for growth irregularities. If any marked changes developed, the experiment would be concentrated on the affected species. Although any growth irregularities that might appear as a result of a low ambient magnetic field could take months or years to develop, this experiment was time limited to the study of possible short term effects.

Proper application of the bacteria to the petri dishes (or "streaking" the plate) was important to prevent contamination from the air or splattering of the innoculation bacteria within the dish. The procedure used was as follows: A wire innoculation loop was first sterilized by heating it in the flame of a Bunsen burner. The lid of the test tube containing the active bacteria culture was removed and the top of the test tube flamed. The sterilized wire loop was dipped into the bacteria culture, then the top of the test tube was again flamed before replacing the lid. Next the lid of the petri dish was lifted slightly, the tip of the wire loop run across the agar and the lid replaced. The tip of the wire loop was heated again to kill any remaining bacteria. This process was repeated for each of the twelve species of bacteria. Three species were grown in each dish in order to stretch the limited supplies available (figure 8). This process took patience and time, since the wire loop occasionally dug into the agar, splattering bacteria and chunks of agar around the dish. Each petri dish as labeled on the bottom with the species names, then 4 dishes (containing a total of 12 different species) were stacked and taped together. Taping them together slowed evaporation of the agar and prevented accidental spillage or contamination from tipover. The names and common appearances of each of the bacteria species are given in TABLE 1.

Six stacks of petri dishes were prepared with bacteria. Three of these were placed on the middle shelf of the field free space (since the middle shelf had the lowest field intensity) as shown in figure 9. The remaining three "control" sets were placed outside of the Helmholtz system on a desk approximately 10 feet away where the ambient geomagnetic field was "normal" as determined by a previous magnetic survey of the lab.

Thermometers were placed near the dishes to monitor temperature variations, and daily recordings were made. The temperature was difficult to control as the heat was uncomfortably high throughout the Mendenhall laboratory building during the winter months. However, the high heat could be controlled somewhat by using window fans. The temperature variation averaged about 2°F between the field and control specimens. During the four week span of this phase of the experiment, the following temperature variations were noted:

	Average	High-Low
Field	79.0°F	73-85°F
Control	77.9°F	72-84°F

Although each petri dish contained many generations after a few days growth, the writer used the term "generation" in the sense that each time the bacteria was transferred to a new dish, a new generation was produced. The first generation showed no noticable growth irregularities. Two species, C. violaceum and P. aeraginosa, showed no growth in any of the dishes. The other ten active cultures were transferred to new petri dishes to begin a second generation. The growth from these dishes showed no irregularities with daily examinations.

After these results were obtained, Dr. Dugan suggested starting again and checking the dishes more often. Six new sets of dishes (eleven species each set) were started from a single set of control dishes. Enough C. violaceum did finally grow in the control dish for use in this phase of the experiment. The P. aeruginosa still showed no evidence of growth in any of the dishes.

During the second generation of the new growth two of the three Acinetobacter colonies in the Helmholtz system developed a mottled appearance. Bacteria from the mottled areas and from the control dishes were transferred to new dishes while growth of all other species was discontinued. These dishes were streaked in a slightly different manner (figure 10) in order to allow for a more critical examination of the growth patterns. After the fourth generation of new growth, the same mottled appearance developed in the control dishes. Since the mottled appearance developed on both the control and test "bacteria", it was probably a result of temperature variations, as Acinetobacter is very temperature sensitive.

The first phase of this experiment failed to show any obvious effects that a low magnetic field might have upon microorganisms on a short term basis. A more precise method of growth measurement was needed. One such method would be to grow an organism in a liquid medium, then measure the turbidity (or absorbance) of the liquid with a mass spectrometer. This would allow a more precise, though indirect, measurement of relative growth rates.

PHASE II

The species selected for this second phase of the experiment was Chlorella vulgaris, a single-celled, chlorophyll producing algae which was readily available from the microbiology laboratory.

Six 250 ml glass flasks were chosen to hold the algae solutions. Each flask was filled with 100 ml of Allen's medium (table 2), stoppered with foam rubber corks and autoclaved at 200°C for 15 minutes to ensure sterilization. After cooling, each flask was innoculated with 1 ml of a Chlorella vulgaris

suspension using a sterile glass pipette. Standard sanitary procedures were used to prevent possible contamination of the solutions.

A light source was required but had to be placed outside the Helmholtz system to avoid metallic interference in the field free space. To do this, two gooseneck lamps were equipped with 150 watt outdoor spotlights. Porcelain extension sockets were used to prevent overheating of the standard lamp sockets. Three of the flasks were placed on the middle shelf in the field free space (figure 11). The spotlight was positioned exactly six feet away. The remaining three "control" flasks were placed on a counter top with the second gooseneck lamp also positioned six feet away (figure 12). Because a foil reflector was in place in the Helmholtz system to insulate the shelves from the furnace, a similar foil reflector was placed the same distance behind the control flasks. The lamps were left on continuously and a thermometer was placed next to each set of flasks to monitor temperature variations. To ensure an adequate supply of carbon dioxide in the solutions, each flask was agitated by shaking twice daily during the first 3 weeks. Temperature readings were taken and recorded each day.

The growth of the algae was slower than anticipated in all flasks, so the experiment was continued for a fourth week. Due to the writer's busy academic schedule, the flasks were not agitated during the fourth week nor were temperature readings taken. However, all flasks were subjected to similar light and temperature conditions. The temperature averaged 83.1°F around the control flasks and 83.0°F around the experimental flasks during the first three weeks. After four weeks, enough growth had occurred to begin measurements.

Because measurements would be made while the solutions were in test tubes, the test tubes had to be checked for optical uniformity. Each test tube was checked visually for imperfections, then carefully cleaned. Then each test tube was passed through a mass spectrometer to pick up minor imperfections invisible to the naked eye, but which could affect the experimental results. Imperfect test tubes were returned to supply. Next, one experimental and one control flask were selected and thoroughly vortexed. Ten ml of solution from each flask was poured into separate test tubes. Each test tube was vortexed again before insertion into the mass spectrometer. Measurements were made for absorption of light by Chlorophyll A (wavelength = 680nm) and the major carotenoids (wavelength = 450nm). This gave a measurement of the amount of each substance and thus the relative growth rates of the algae. The following readings were recorded:

PERCENT ABSORPTION

	Chlorophyll A	Carotenoids
control flask	37%	26%
field flask	34%	24%

The slight variations in these readings were considered too small to indicate any substantial changes in growth rates. Such differences could result from a number of factors including slight temperature variations or carbon dioxide availability, but might be shown significant in a later study where particular attention is paid to this aspect of the experiment.

The Chlorella growth was continued for an additional ten days in the remaining flasks without further agitation. Two of the flasks were accidently contaminated so the contents were discarded. The remaining flasks were measured as before and the following readings taken:

PERCENT ABSORPTION

	Chlorophyll A	Caroteniods
control flask	81%	90%
field flask	81%	89%

Again, no significant differences in growth rates were observed between the algae grown under normal conditions and that grown in the field free space.

PHASE III

It is possible that a low ambient magnetic field would somehow have a more direct effect upon an organism that oxidizes iron as part of its metabolic process than on those already studied in this experiment. Such an assumption was the basis for the third and final phase of this experiment. The bacterium chosen for this final phase was Thiobacillus ferroxidans, an iron oxidizing species commonly found in areas of high acid conditions such as mine runoff. Growth rates could be measured by periodically placing samples of the cultures in a diluted HCl solution, killing the bacteria and causing them to settle to the bottom as sediment. The Fe^{+3} ions left suspended in the solution could them be measured using a mass spectrometer in the same manner as before.

A great deal of preparation was necessary for this phase of the experiment. The liquid growth medium selected was 9K medium which has a pH of 3 (table 3). An advantage of using this solution was that normal sterilization procedures were not

necessary since few other organisms could tolerate such an acidic environment.

Because this species required substantial amounts of oxygen for growth, an aeration apparatus was needed to ensure an adequate air supply. Four flasks would be aerated at once, two within the Helmholtz coils and two outside the field as controls. Air from a central outlet in the lab was usable but required filtration since it contained traces of oil from the air compressor. Filtration was accomplished by passing the air through water in a half full plastic jug (figure 13). After filtration the air flow was divided with a "T" fitting, with one hose extending to the flasks in the Helmholtz system and the other to the control flasks. Each hose was split again with a T-fitting, then connected to the appropriate flask. Each flask was equipped with a twin hole rubber stopper. The inlet hole was configured with a Pasteur pipette (a glass tube with one end drawn out to capillary size) and hooked up to the rubber air hose. The second hole was equipped with a standard glass tube to act as an exhaust (figure 14).

In order to maintain uniformity, a method was needed to equalize the air flow into each flask. This was accomplished by attaching a hose clamp to each rubber tube at a point above each flask. The air flow was then measured by placing the hose/pippette hookup for each flask in a one liter flask filled with water and inverted in a larger bucket of water. The air was turned on and the hose/pippette apparatus placed in the inverted, water filled flask. The time needed for the air to completely displace the water in the 1 liter flask was recorded. This was done for each hose/pippette apparatus, and the hose clamps adjusted to equalize the air flow through each hose. As one might imagine, each time an adjustment was made to one clamp, the air flow through the other hoses had to be measured again and readjusted. This process was repeated numerous times until the "displacement times" for the four pippettes were within seven seconds of each other. Further "fine tuning" would have required a more sophisticated system. The final adjustments yielded the following:

Time required to displace 1 liter of water

hose #1 (Helmholtz field)	70 seconds
hose #2 (Helmholtz field)	68 seconds
hose #3 (control)	73 seconds
hose #4 (control)	75 seconds

After the air flow adjustments were made, four flasks were selected and each was filled with 150 ml of 9k medium (table 3). Two of these were innoculated with 0.5 ml of an active Thiobacillus ferroxidans culture. The other two flasks were not innoculated, since the iron in the 9K medium would still oxidize

without the bacteria, but at a slower rate. These two flasks served as controls. One of each flask, innoculated and control, was placed on the middle shelf in the Helmholtz system (figure 15). The remaining two flasks were placed in the normal lab geomagnetic field.

After several days a rusty color began to appear in the flasks (evidence of oxidation). Two 1.0 ml samples were taken from each flask and placed in separate test tubes containing 9.0 ml of 1.1N HCl, yielding 10 ml of a 1N solution. (Having a 1N HCl solution was part of an original plan to compare the results of this experiment with a standard curve for growth of Thiobacillus, but conditions of growth were too different to allow such a comparison.) The bacteria were killed immediately upon exposure to the HCl and settled to the bottoms of the test tubes as sediment. The relative concentrations of Fe^{+3} ions left suspended in the solution were then determined by measuring for absorbancy with a mass spectrometer. The results of each pair of test tubes were averaged and recorded. This process was continued periodically until the oxidation process appeared to reach a peak. The results were tabulated (table 4) and plotted on a graph for comparison (figure 16).

As expected, there was a definate difference in growth rates between the flasks innoculated with bacteria and those containing only 9K medium. Also noted were variations between the solutions grown inside and outside the Helmholtz coils. This variation was almost negligable for the flasks containing the bacteria. However, a greater difference in oxidation rates developed between the flasks that were not innoculated with bacteria. This difference may have been due to uneven airflow into the flasks, but additional study of this observation could be included in any future study of the subject. In retrospect, more accurate results could have been obtained had an amino acid buffer been used in the solutions to stabilize the pH levels. This should be considered as an experimental constant in any future work.

CONCLUSION

This project was the first in depth experiment undertaken by the writer. It seems that more was learned about "how not to" than "how to" do things. Yet the data obtained is accurate enough to conclude that, in the particular cases studied, no significant growth irregularities were induced as a result of the bacteria and algae growth in a low ambient magnetic field.

The results of this experiment apply to observations of short term growth only. It is possible under the conditions provided that growth irregularities might appear on a longer term basis. It is also possible that only certain select organisms would be affected. A study over a longer period of time than was possible for this experiment would be appropriate, with the services of a full time microbiologist essential. In the event that a longer term study is attempted, it should be remembered that some changes might take place due to normal mutations. Greater care should also be taken to eliminate as many variables (such as contamination, temperature variations, etc.) as possible to make the results more accurate.

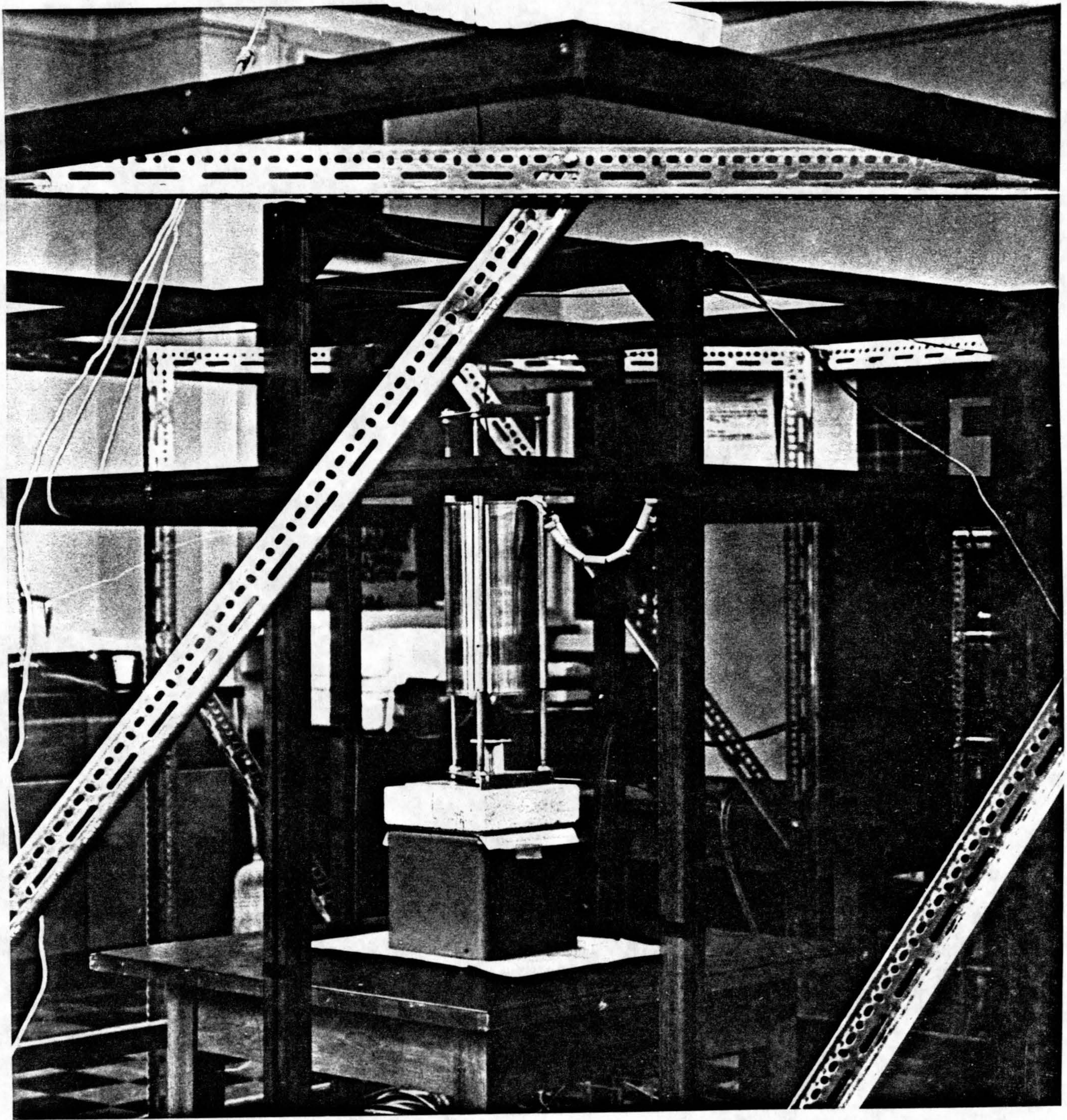

FIGURE-1 HELMHOLTZ CORE APPARATUS

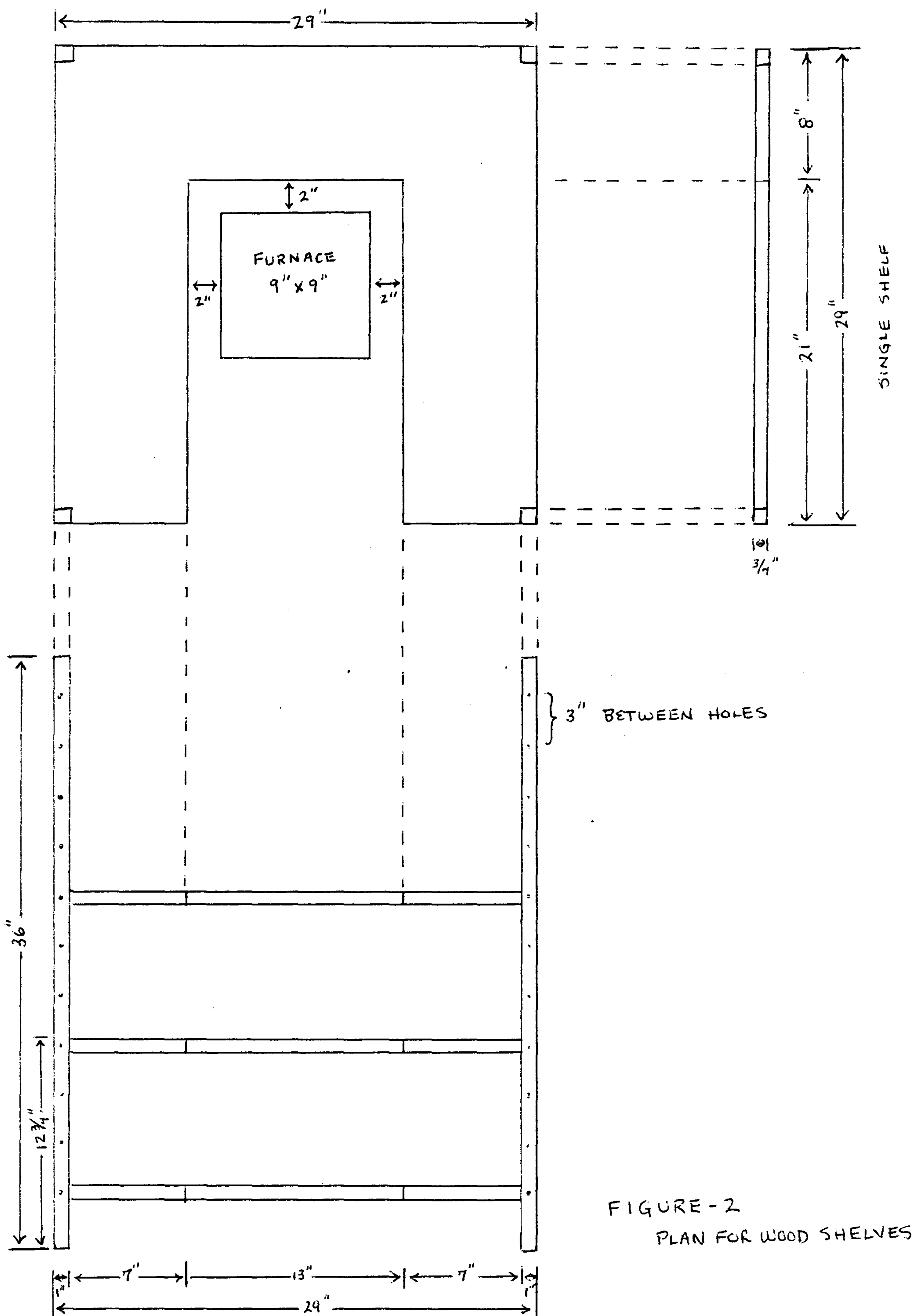

FIGURE - 2
PLAN FOR WOOD SHELVES

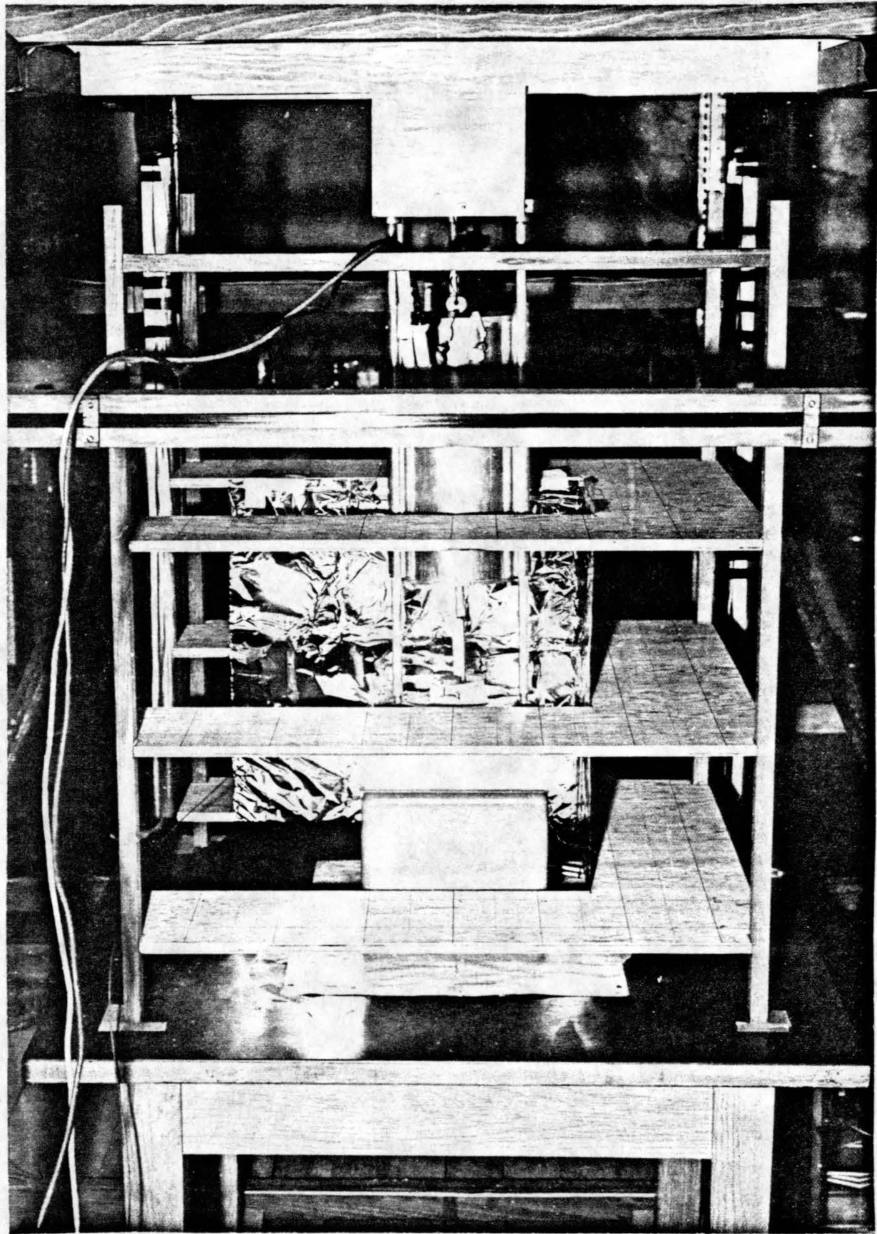

FIGURE 3 - WOODEN SHELVES

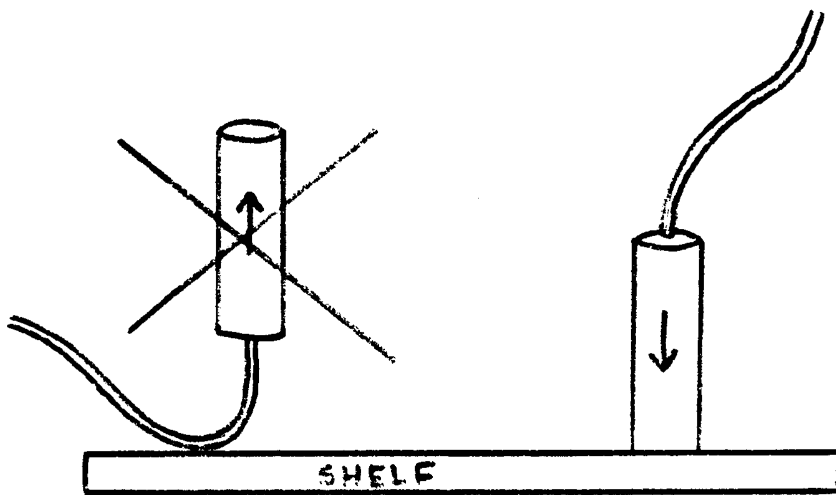

FIGURE 4 - MILLIGAUSS METER PROBE ORIENTATION

TOP SHELF

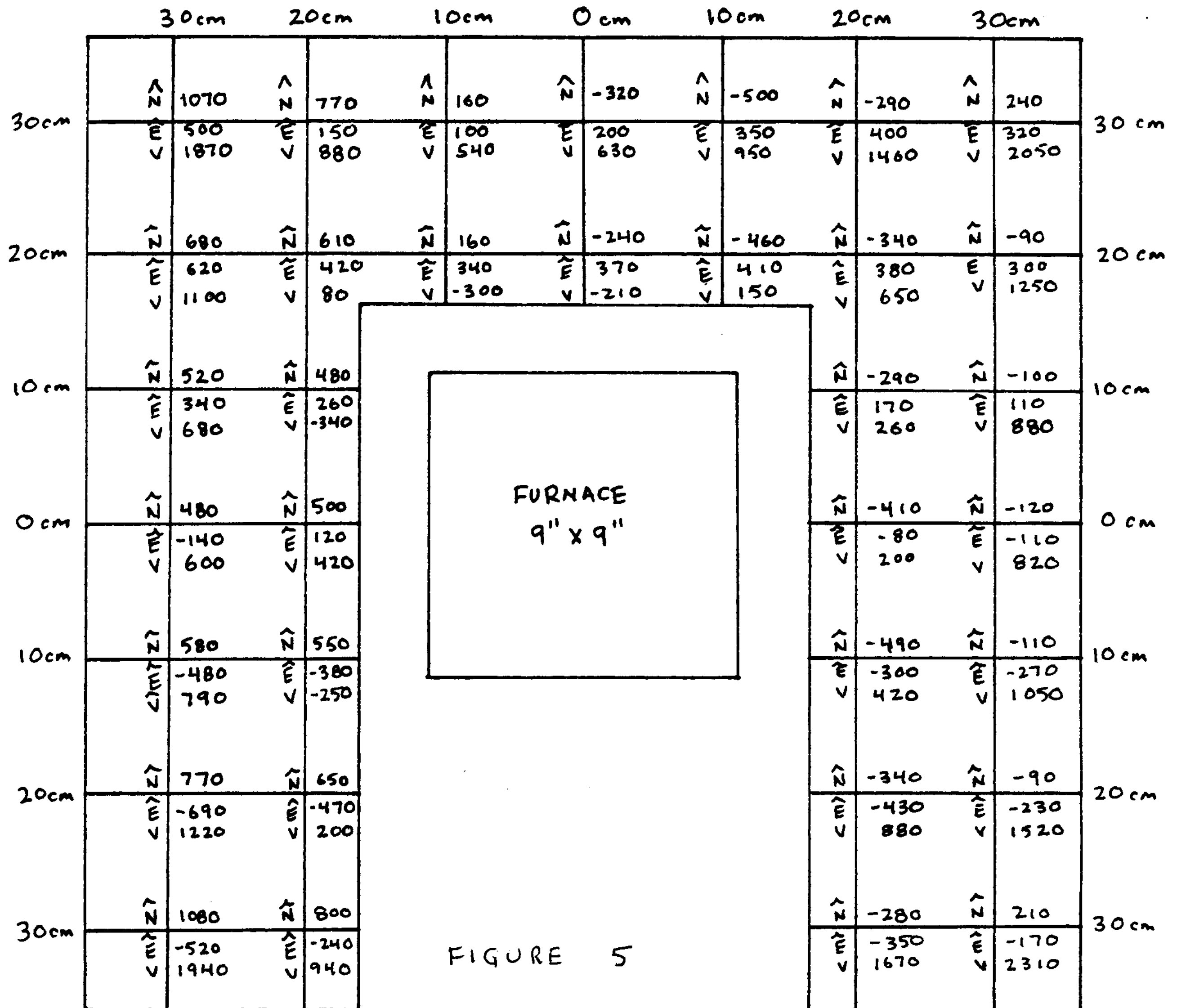

FIGURE 5

MAGNETIC FIELD INTENSITIES

- MEASUREMENTS IN GAMMAS
- MEASUREMENTS MADE WITH SCHONSTEDT MILLIGAUSS METER
- GRID DIVISIONS IN CENTIMETERS FROM CENTER OF FURNACE
- SCALE 1:5
- ESTIMATED ERROR = 1 MILLIGAUSS or 100 γ
- ^ INDICATES AVERAGE

MIDDLE SHELF

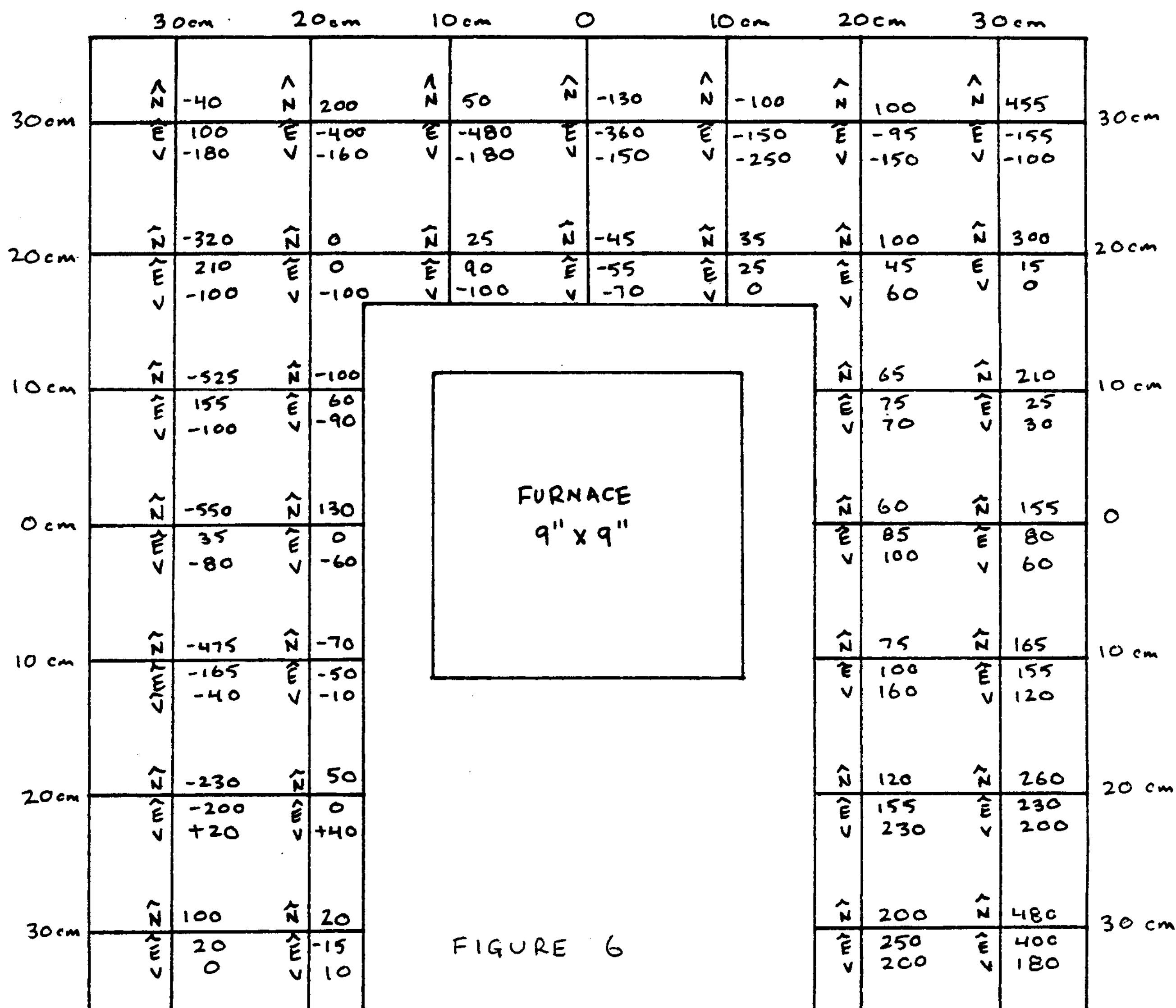

FIGURE 6

MAGNETIC FIELD INTENSITIES

- MEASUREMENTS IN GAMMAS
- MEASUREMENTS MADE WITH SCHONSTEDT MILLIGAUSS METER
- GRID DIVISIONS IN CENTIMETERS FROM CENTER OF FURNACE
- SCALE 1:5
- ESTIMATED ERROR = 1 MILLIGAUSS or 100 γ
- ^ INDICATES AVERAGE

BOTTOM SHELF

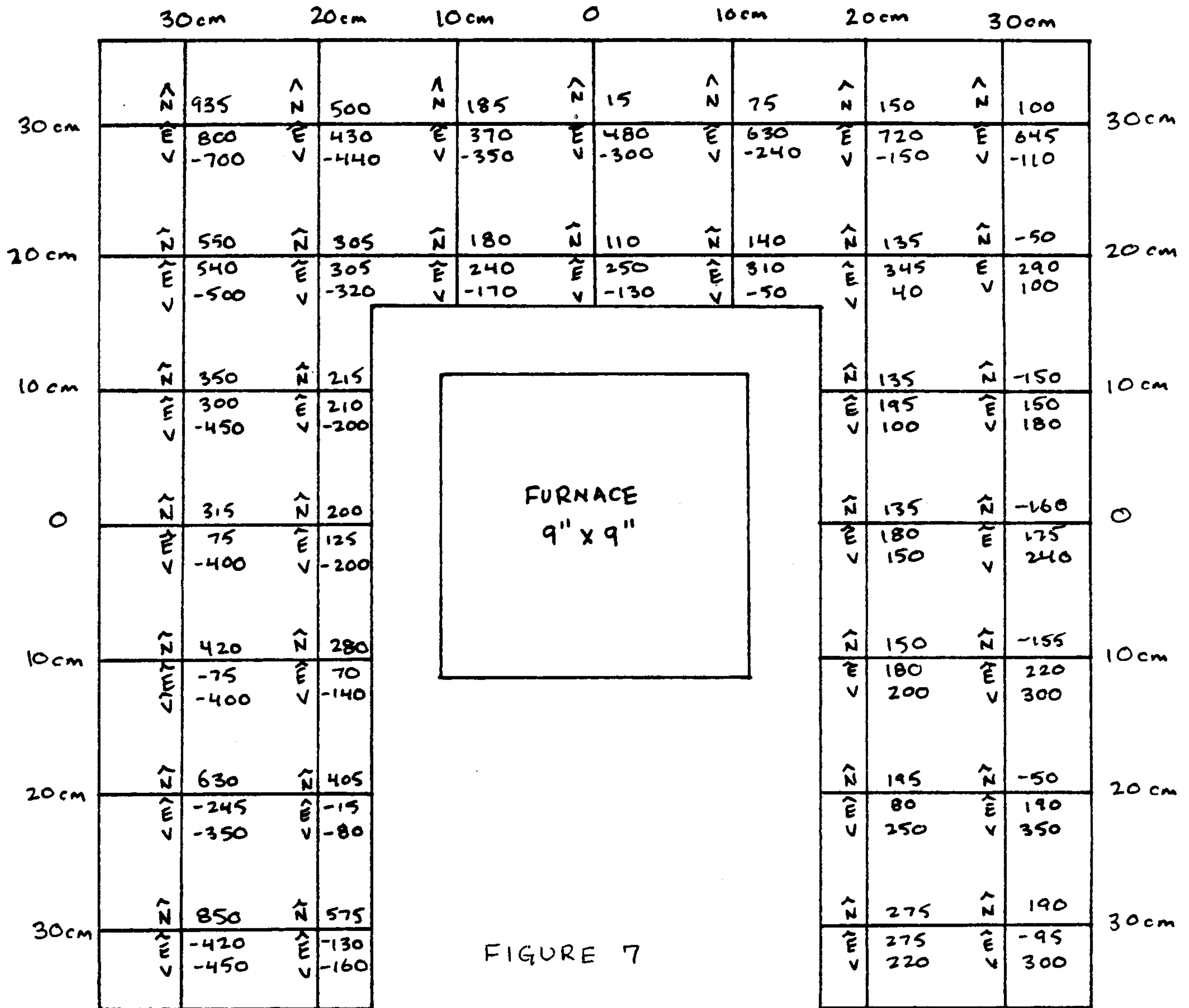

FIGURE 7

MAGNETIC FIELD INTENSITIES

- MEASUREMENTS IN GAMMAS
- MEASUREMENTS MADE WITH SCHONSTEDT MILLIGAUSS METER
- GRID DIVISIONS IN CENTIMETERS FROM CENTER OF FURNACE
- SCALE 1:5
- ESTIMATED ERROR = 1 MILLIGAUSS or 100γ
- ^ INDICATES AVERAGE

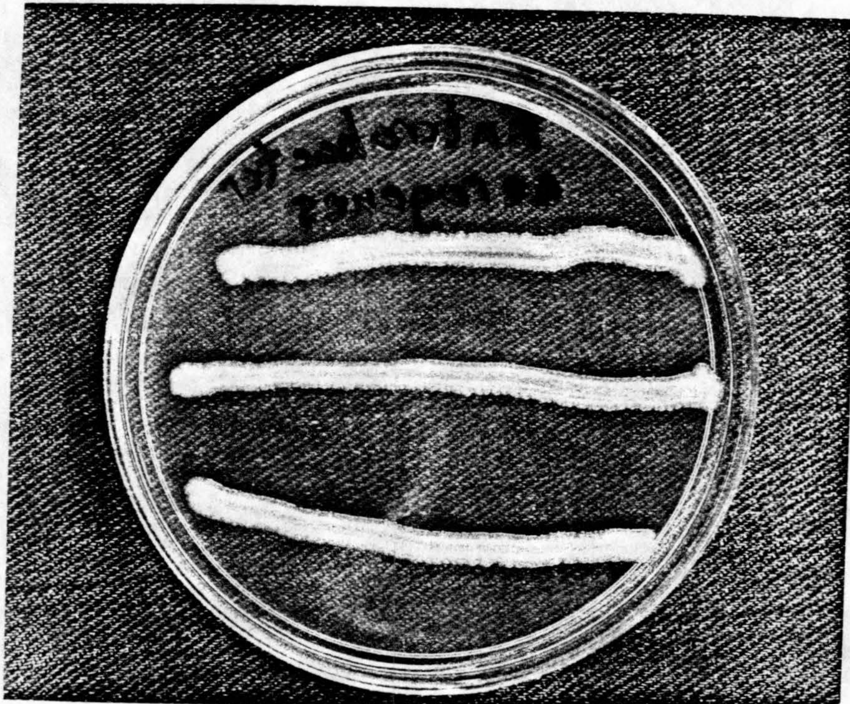

Figure 8 - 3 species of bacteria

Colony No.	Name	Color
889	Acinetobacter	white
313	Enterobacter cloaceae	white
234	Serrita marcescens	pink (20-25°C)
167	Pseudomonas aeroginosa	fluorescent green
224	Shigella pullorum	---
40	Flavobacterium suavealens	yellow
44	Chromobacter violaceum	violet
120	Alcaligeres faecalis	white
48	Steptococcus faecalis	white
303	Staphylococcus aureus	gold
108	Xanthamonas campestris	yellow
395	Escherichia coli	white

Table 1 - Bacteria species

PETRI DISH PLACEMENTS

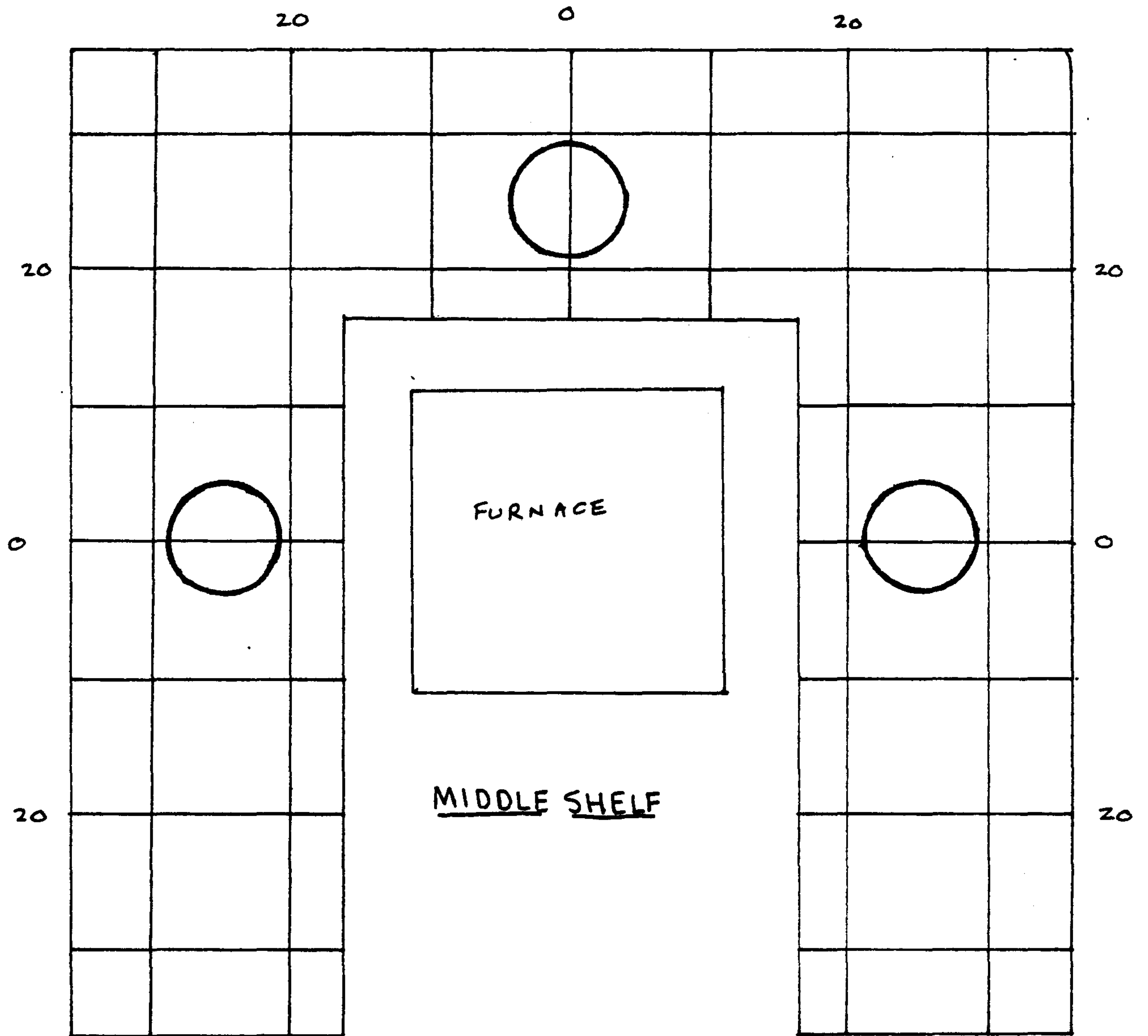

FIGURE 9

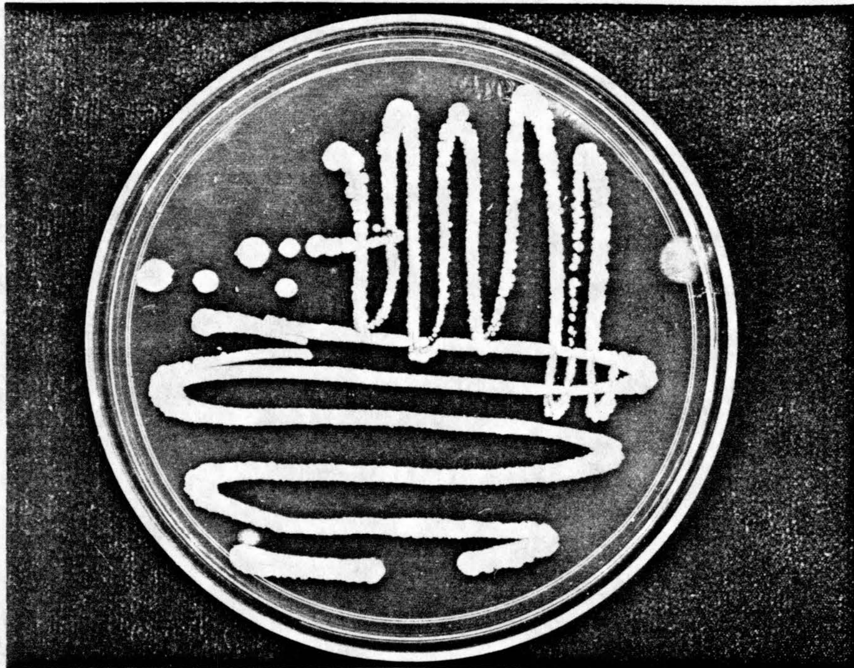

Figure 10 - Single species growth

<u>Allen's</u> <u>medium</u> (Allen, 1952)	
NH_4Cl	50 mg
$NaNO_3$	1000 mg
K_2HPO_4	250 mg
$FeCl_3$	3 mg
$MgSO_4 \cdot 7HOH$	513 mg
$CaCl_2$	50 mg
plus distilled H_2O to 1 liter	

Table 2

PLACEMENT OF FLASKS CONTAINING
CHLORELLA VULGARIS

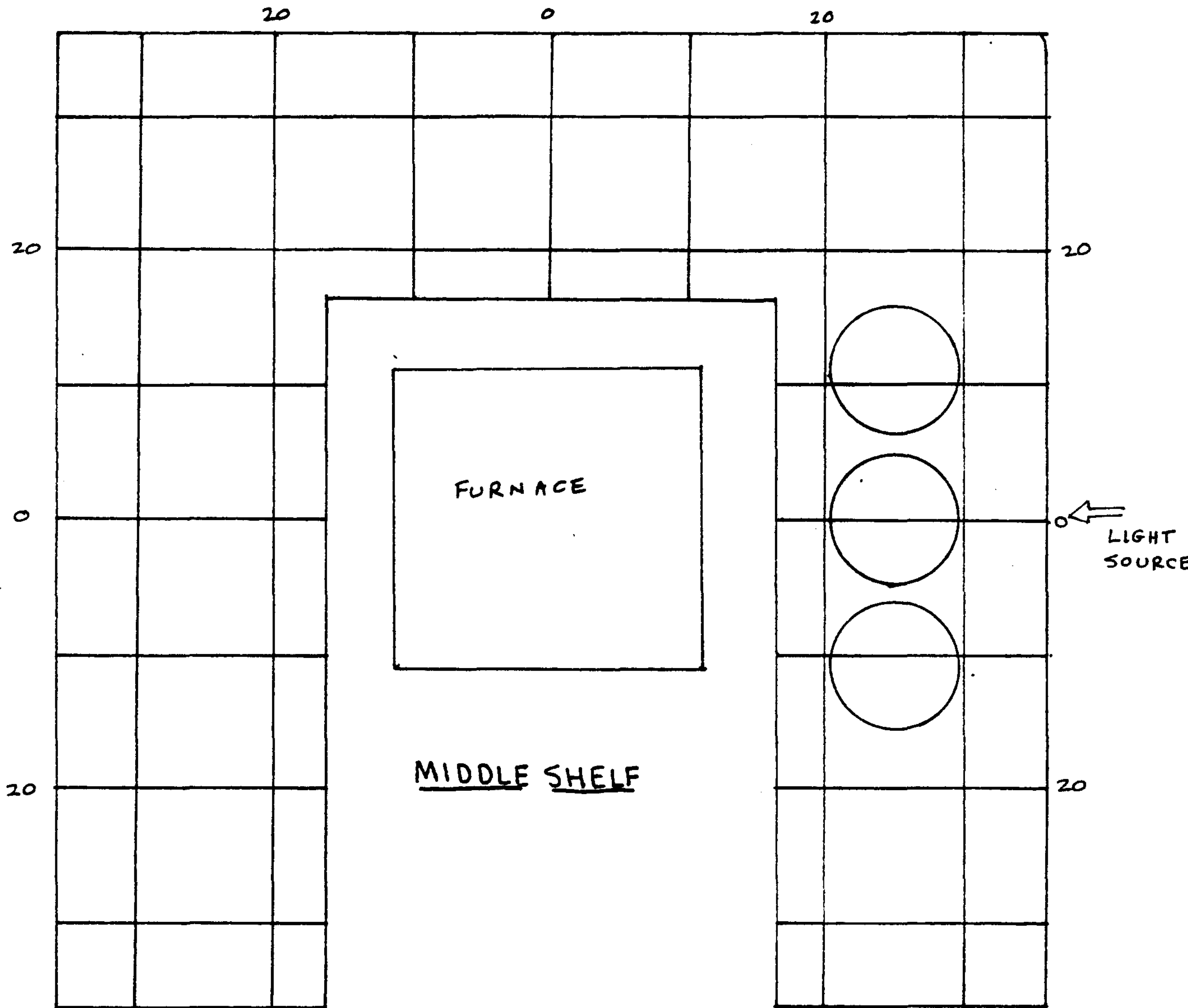

FIGURE 11

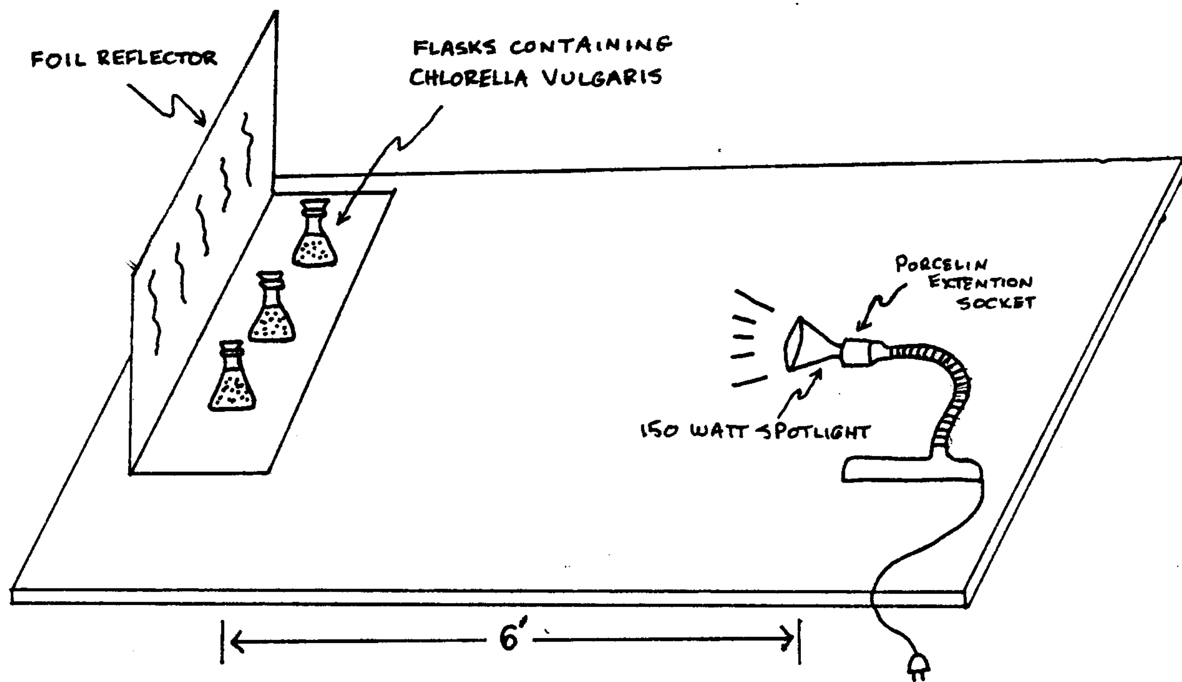

FIGURE 12 — APPARATUS FOR GROWTH OF CHLORELLA VULGARIS

<u>9K</u> <u>medium</u> (Silverman & Ludgren, 1959)

$(NH_4)_2SO_4$	3.0 gm
KCl	.1 gm
K_2HPO_4	.5 gm
$MgSO_4 \cdot 7HOH$	.5 gm
$Ca(NO_3)_2$	.01 gm
$FeSO_4 \cdot 7HOH$	44.2 gm
10N H_2SO_4	1.0 ml

plus distilled water to 1 liter

Table 3

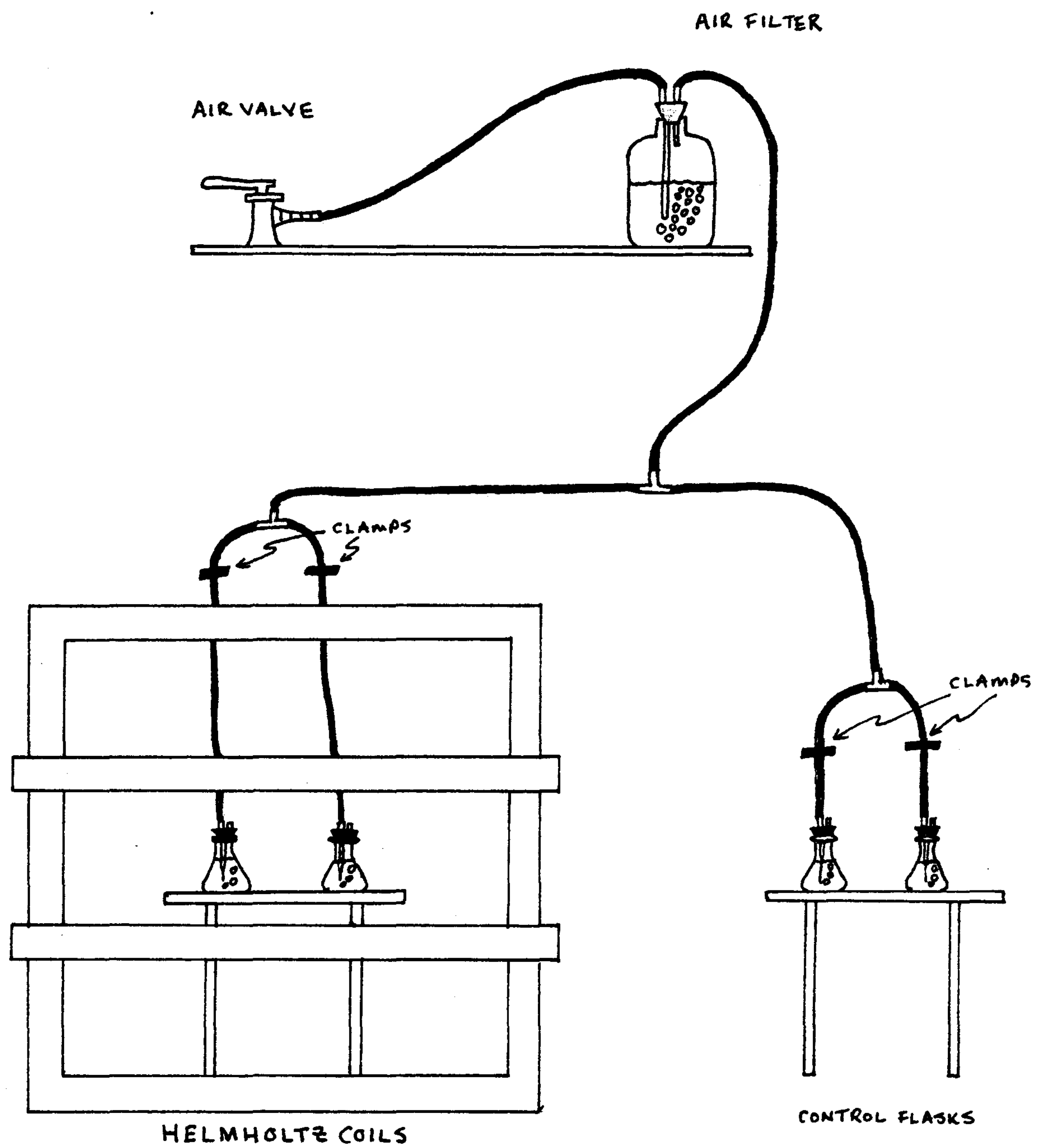

FIGURE 13

SIMPLIFIED SKETCH OF APPARATUS FOR GROWTH of THIOBACILLIUS

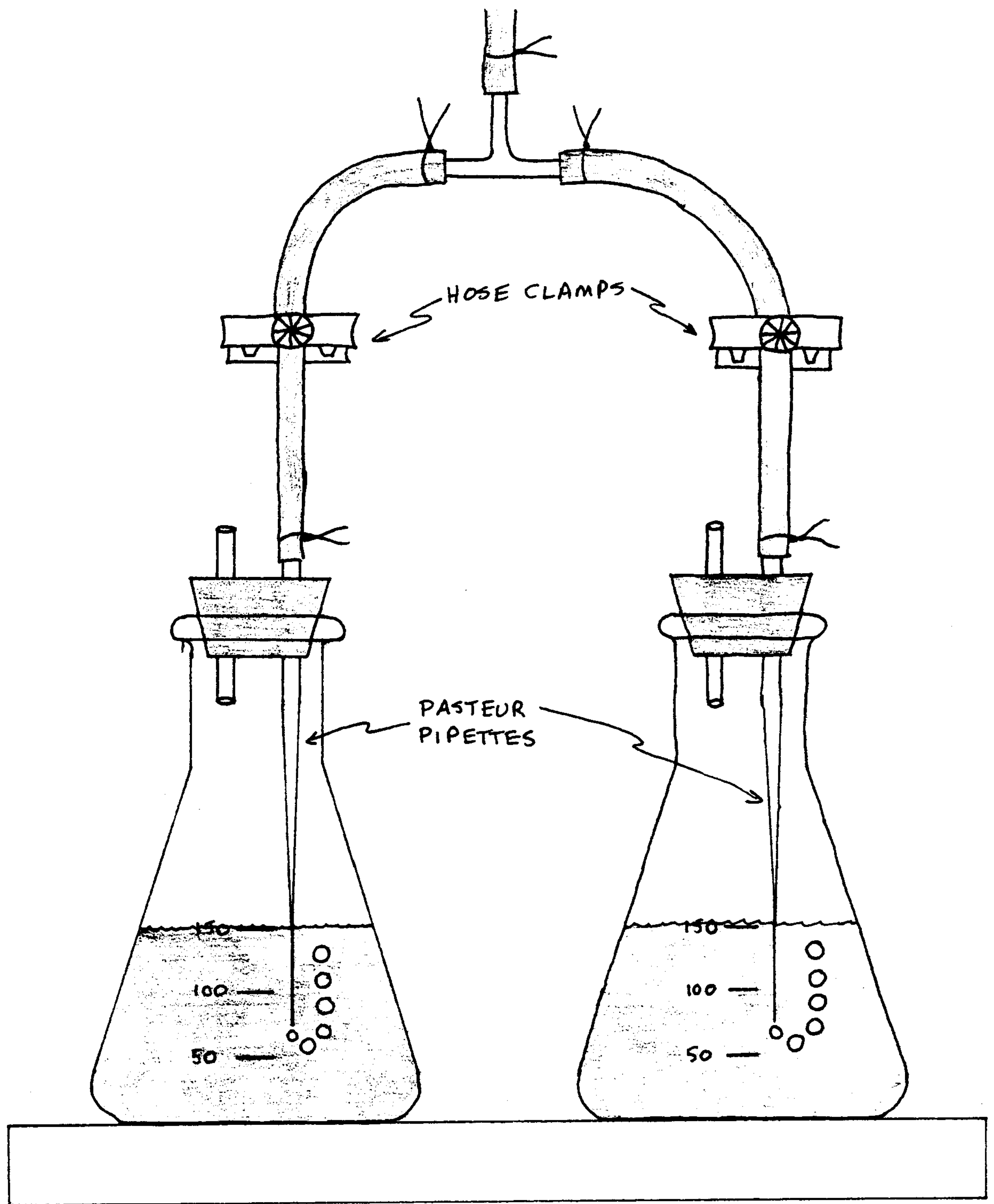

DIAGRAM OF FLASKS USED FOR GROWTH OF THIOBACILLIUS BACTERIA

FIGURE 14

PLACEMENTS OF FLASKS CONTAINING
THIOBACILLIUS FERROXIDANS

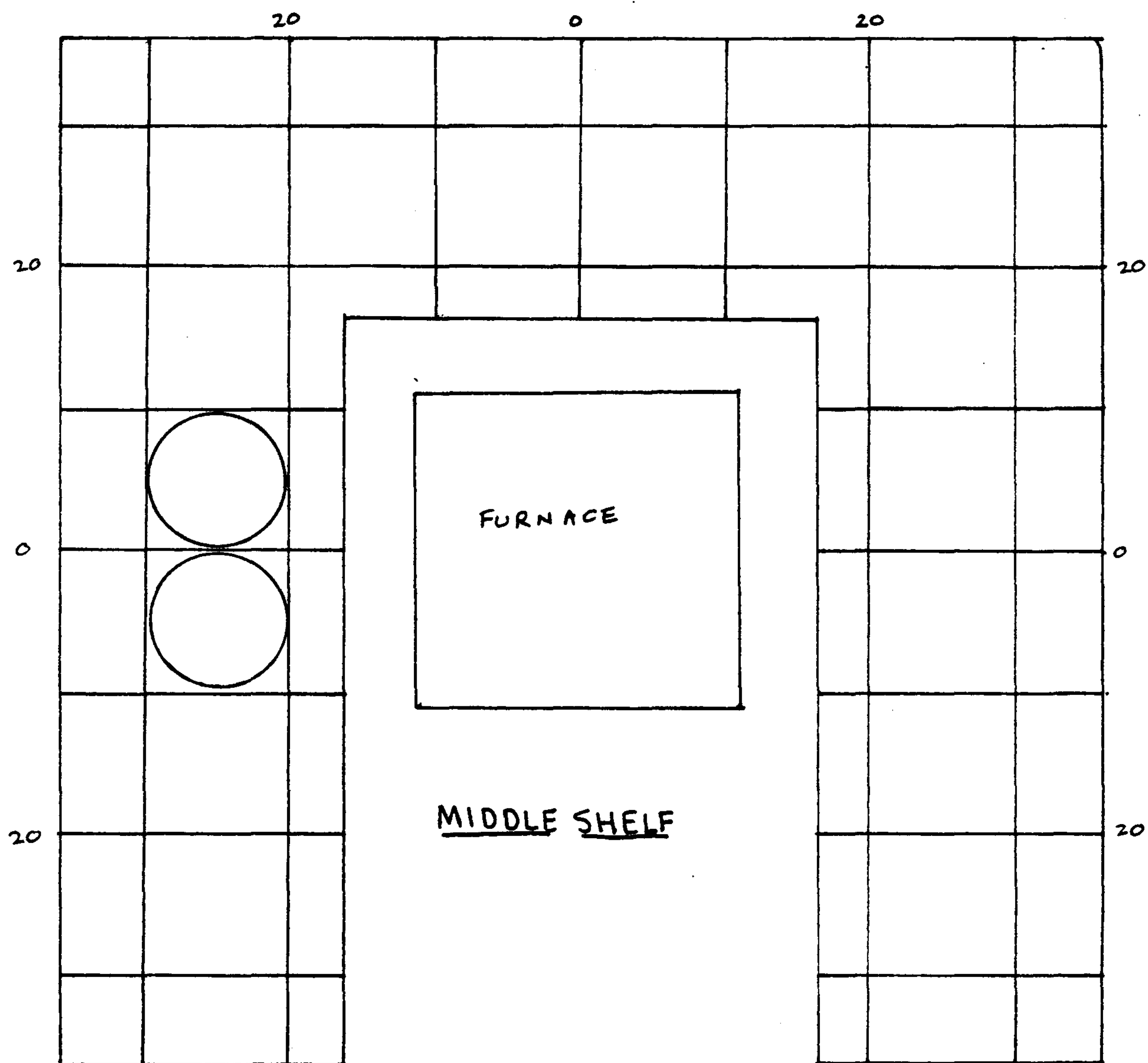

FIGURE 15

% Absorbance

Sample #	Hours of Growth	Cc	Fc	Ci	Fi
1	35	4%	3%	17%	13%
2	46	7%	6%	28%	26%
3	57	9%	7%	58%	50%
4	77*	14%	11%	91%	89%
5	125	26%	19%	91%	89%
6	143	64%	41%	91%	89%

* visible precipitate formed

Cc = uninnoculated flask in normal magnetic environment

Fc = uninnoculated flask in Helmholtz

Ci = innoculated flask in normal magnetic environment

Fi = innoculated flask in Helmholtz

Table 4 - Oxidation rates of Thiobacillius

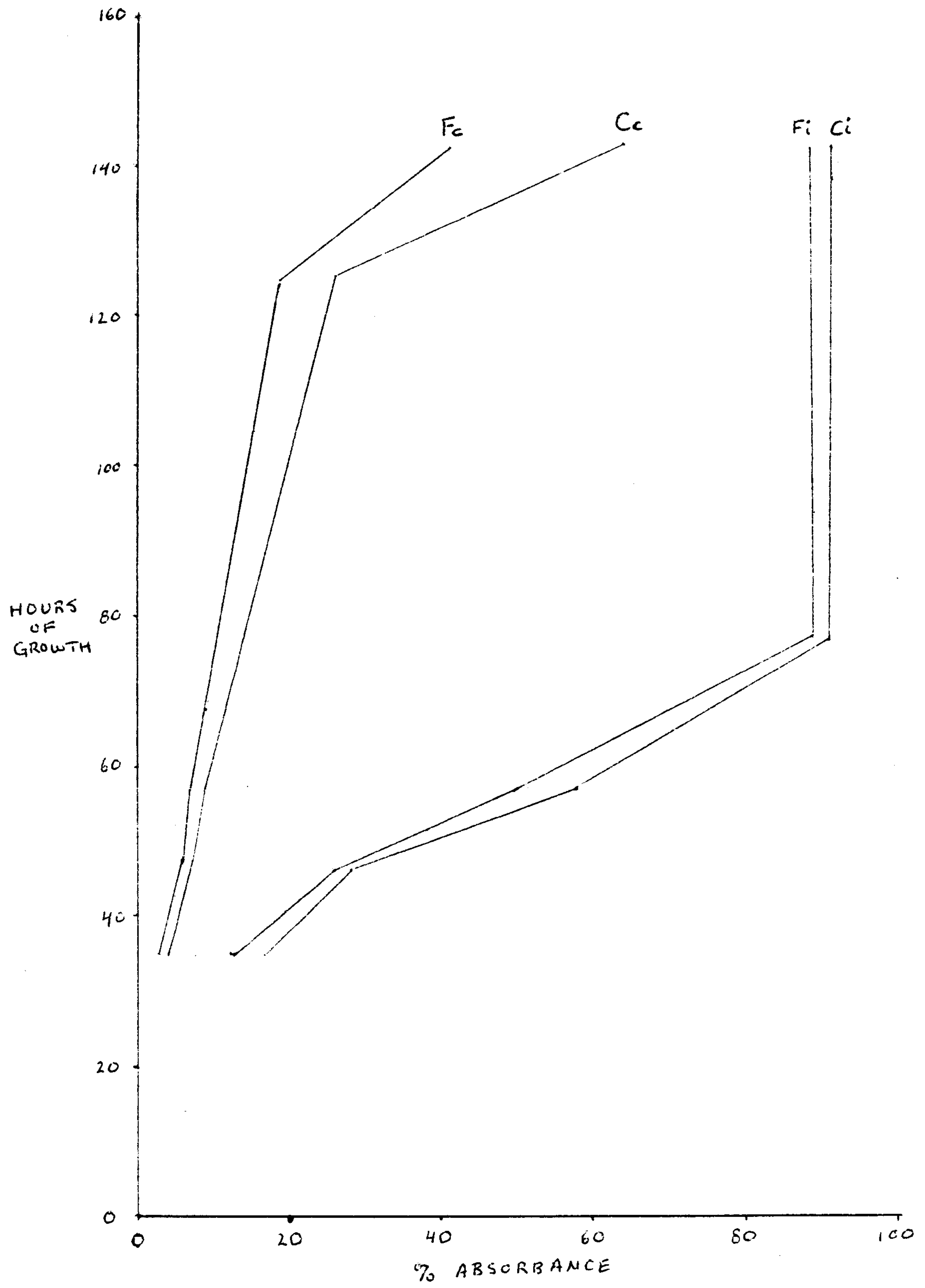

FIGURE 16 - THIOBACILLIUS GROWTH

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