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I. Auximones and the growth of the green plant; II. Organic matter and the growth of Lemna

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I. AUXIMONES AND THE GROWTH OF THE GREEN PLANT.

II. ORGANIC MATTER AND THE GROWTH OF LEMNA.

BY

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I. AUXINONES AND THE GROWTH OF THE GREEN PLANT.

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"AUXIMONES" AND THE GROWTH OF THE GREEN PLANT

BY

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"AUXIMONES" AND THE GROWTH OF THE GREEN PLANT[1]

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The possibility of the presence in the soil of growth-promoting accessory substances is still an open question. The nutrition of the green plant has generally been regarded as satisfied by a supply of inorganic food materials, consisting of mineral matter, carbon dioxide and water, from which organic material is produced and the complete plant structure built up. Recently, however, this conception has been questioned by Bottomley (1–6) and Mockeridge (15) who have stated that the addition of minimal quantities of certain organic substances to the inorganic nutrients is absolutely essential if the plants are to grow healthily and normally for any length of time.

To these organic substances Bottomley gave the name *auximones*, and it was suggested that they were similar in function to the growth-promoting accessory factors termed vitamines, necessary for the growth of animals. "It is now established that plants, in their turn, require growth-promoting substances, or auximones; which, in the case of the lower plants, are apparently manufactured by themselves, but which in the case of green plants, must be supplied from without. Since these necessary accessory substances are essentially organic in nature, their only possible source in the case of ordinary green plants is to be found in the organic matter of the soil in which they are growing."

Several patents were secured by Bottomley for the manufacture of these growth-promoting substances from bacteria-treated peats, and an effort was made to use the *auximones* on a commercial scale in both greenhouse and market garden practise. A popular account is given by G. D. Knox in *The Spirit of the Soil* and marked success is reported in many cases.

In order to avoid introducing the *auximone* in the seed, Bottomley worked in the laboratory principally with *Lemna minor* and *Lemna major* which reproduce by budding, using Detmer-Moor's and Knop's solutions (6). With these solutions he failed to get good growth and after a few weeks both the appearance and weight of the *Lemna* indicated that the plants were not receiving all that they required to maintain their normal health. Mockeridge (15) had the same experience with *Lemna major*. In both cases the addition of small quantities of organic matter, extracted from soil or from the specially treated peat, greatly increased the rate of reproduction and the health of the plants.

Mockeridge has quoted the work of Williams (20) who arrived at the conclusion that a substance identical with the water-soluble vitamine B was necessary for the growth of yeast. The experience of one of the writers with *Saccharomyces cerevisiae* (7) and recent work in these laboratories by Fulmer (9) and Nelson (16) as well as reports by McCollum and his co-workers (13) have shown that the accessory substance for yeast, the *Bios* of Wildiers (19), is of the nature of an accelerator rather than an essential for reproduction and cannot be compared with animal vitamines without which reproduction and growth cannot take place. Robertson

[1] Contribution from the Department of Chemistry, Iowa State College, Ames, Iowa.

(17) has suggested that this may be the same *substance X* which he finds accelerates the reproduction of infusoria.

It seemed possible therefore, that the function of organic matter in the nutrition of green plants might be to increase the speed of reproduction and growth, rather than serve as an essential constituent, and that the reason for poor growth in Knop's and Detmer's solutions might be the unsuitability of the media used. Bottomley (6) recognized this when using Detmer's solution alone: he therefore tested Knop's and found that it checked his results with Detmer's, but he does not seem to have tried others. Mendiola (14) in a genetic study of *Lemna minor*, reported successful growth and even increased size of plants with a modified Pfeffer's medium which contained no organic matter.

EXPERIMENTAL

After preliminary experiments *Lemna major* was chosen and the three salts, monocalcium phosphate, potassium nitrate and magnesium sulfate, were used as a basis of the medium. These salts were first tested by Livingston and Tottingham (12) for wheat. Iron was added to the solutions as ferric phosphate and was made up as described in the Plan for Coöperative Research of the National Research Council (11); approximately 0.38 mgm. of $FePO_4$ were added to 250 cc. of the solutions.

The calcium and magnesium salts were made up in 0.05 *M* solutions at 20°C. and the potassium nitrate 0.20 *M*. A modified Shive's apparatus (18) was used for filling and the plants were grown in Pyrex beakers which were wrapped to the level of the liquid with paper, black inside and white outside, and covered with a watch glass to prevent the entrance of dust. Each beaker contained 250 cc. of solution and this was changed twice a week as with Bottomley and Mockeridge: no trouble was caused by algae or mold under these conditions. The beakers were weighed and water lost was replaced each morning; specially redistilled conductivity water was used in all cases—the laboratory distilled water had a decidedly toxic effect on the plants. The stock cultures were grown in soil solutions, 25 gm. of a Carrington loam soil to 200 cc. water, and renewed weekly. All cultures received direct sunshine in the afternoon only.

One hundred and twenty-five solutions were made up, being all combinations of the three salts in the following proportions:

$Ca(H_2PO_4)_2$	KNO_3	$MgSO_4$
moles per liter	*moles per liter*	*moles per liter*
0.0004	0.0016	0.004
0.001	0.004	0.001
0.002	0.008	0.002
0.003	0.012	0.003
0.004	0.016	0.004

RESULTS

Within a week all plants in solutions containing 0.002 mole of monocalcium phosphate had turned brown and were dying or dead. This was not due to excess of calcium or phosphate for when the ions were rearranged, greater amounts were not harmful. On the other hand, 0.008 mole of KH_2PO_4 per liter proved toxic in several combinations. The effect of hydrogen ions is being further studied.

At the end of two weeks, twenty-two cultures which showed the best development with regard to size, color and number were selected for tests on their rate of reproduction. All were healthy and varied little in size from the controls in soil solution—a condition still shown after four months growth in the mineral solutions. The ratio of calcium to magnesium varied from 2.5 to 0.25.

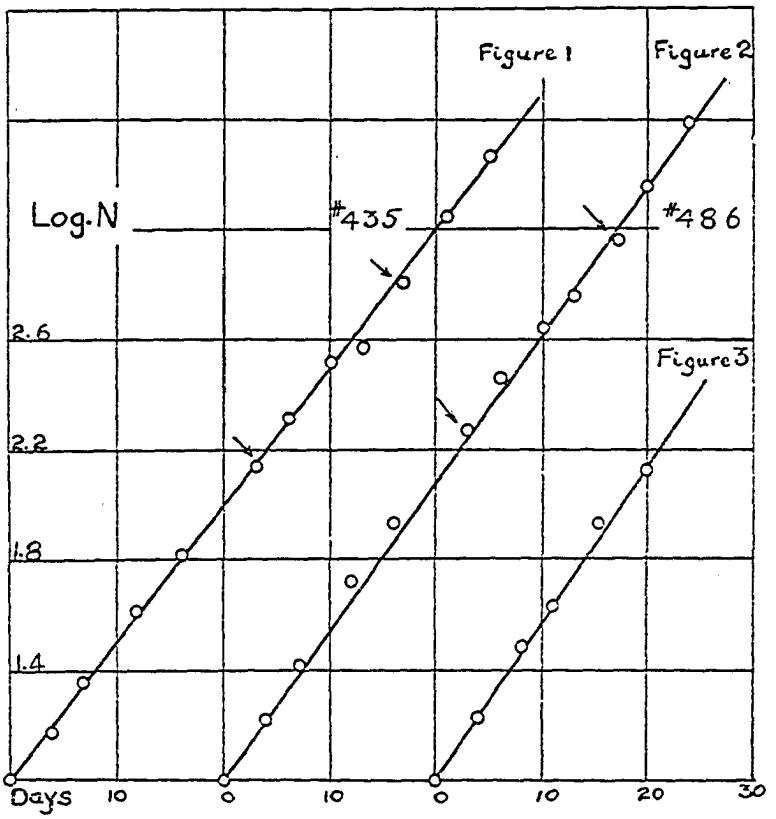
Given good conditions, the rate of increase at any time should be proportional to the number of plants present, whence

$$\log_{10} \frac{N}{N_o} = k\,(t - t_o)$$

If the logarithm of the number of plants is plotted on time, the result is a straight line with slope k. The variation in temperature and sunlight, and possibly the time of the growing season, would be expected to make the curve somewhat irregular, but remarkably uniform results were obtained. Two typical curves are shown in figures 1 and 2, solutions 435 and 486. At the points marked with the arrows the beakers became crowded and the number of plants was reduced to ten or twelve. As will be seen, this did not alter the slope of the curve. Figure 3 shows the soil control, which contained 25 gm. soil and 200 cc. water, changed twice a week as with the mineral solutions. The k for this is 0.058, indicating that a plant reproduced itself—the "generation time"—in 5 days, compared to $5\frac{1}{2}$ to $5\frac{3}{4}$ for the mineral solutions.

In figures 4 and 5 are plotted of Bottomley's curves for Detmer's solution alone and Detmer's plus peat extract (6). In both cases the plants started to reproduce during the first week at a rapid rate and a generation consisted of four to five days. This rate, however, was not kept up but fell to about twenty days for the mineral solution alone and to 11 days for the medium including the peat extract. Upon adding the extract the rate of reproduction was doubled but it did not approach that of the first seven days. Curves for Knop's solutions plotted from both Bottomley and Mockeridge show the same shape but the time is even longer.

No such drop is shown in figures 1 and 2, where the rate of growth continued at twice the speed shown after the first week in Bottomley's medium containing minerals and peat extract. The rate of reproduction shown in figure 1—after growing with sunshine during the afternoon only—was rather smaller than for the plants grown in Bottomley's greenhouse during the first

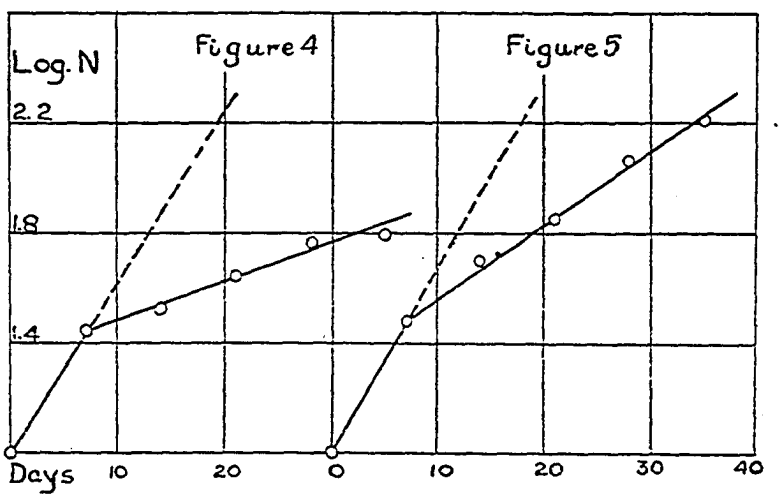


FIGS. 1–3. REPRODUCTION OF *Lemna* IN THREE SALT AND SOIL SOLUTIONS

FIG. 1. Solution no. 435. $Ca(H_2PO_4)_2$ = 0.0004 moles per liter; KNO_3 = 0.0004 moles per liter; $MgSO_4$ = 0.0004 moles per liter. k = 0.051. Generation time = 5.9 days.

FIG. 2. Solution no. 486. $Ca(H_2PO_4)_2$ = 0.001 moles per liter; KNO_3 = 0.004 moles per liter; $MgSO_4$ = 0.002 moles per liter. k = 0.053. Generation time = 5.6 days.

FIG. 3. Soil 25 gm.; water 200 cc. k = 0.057. Generation time = 5.2 days.



FIGS. 4–5. REPRODUCTION OF *Lemna* IN DETMER'S SOLUTION

FIG. 4. Detmer's solution plotted from Bottomley's tables. Generation time for first week = 4.7 days; after first week = 20 days.

FIG. 5. Detmer's solution plus peat extract, plotted from Bottomley's tables. Generation time for first week = 4.3 days; after first week = 11 days.

week in the peat solution, but this rate was almost maintained in the mineral solutions, whereas Bottomley's, even with organic matter present, dropped off rapidly.

It seems therefore, that better growth can be obtained by using a mineral solution suited to the plant than by adding organic matter to one in which the plant has difficulty in developing. For four months, representing over twenty generations, the *Lemna* without *auximones* reproduced at the same rate and the size and health of the plants showed no falling off. The suggestion that *auximones* act as essential constituents for growth of plants, in the same way as the vitamines for animals, must therefore be negatived. The question whether organic matter will function as an accelerator when the solution is adapted for the plant's needs is receiving further study in this laboratory.

SUMMARY

1. The growth of *Lemna major* in mineral solutions depends upon suitable concentrations of salts; organic matter is not necessary.

2. Reproduction, in varying concentrations of $Ca(H_2PO_4)_2$, KNO_3 and $MgSO_4$, with iron supplied as ferric phosphate, attained almost the speed for plants grown in solutions containing soil, and for four months the *Lemna* showed no signs of decrease in size.

3. The number of plants present follow closely the logarithmic equation $\log_{10} \frac{N}{N_o} = k(t - t_o)$. The time for one generation to produce another under the conditions given, for solutions containing soil was five days; for a number of different concentrations of the salts used a generation time of under six days was obtained.

4. *Auximones* are not essential for the growth and reproduction of green plants and cannot be classed with vitamines which are necessary for animal growth.

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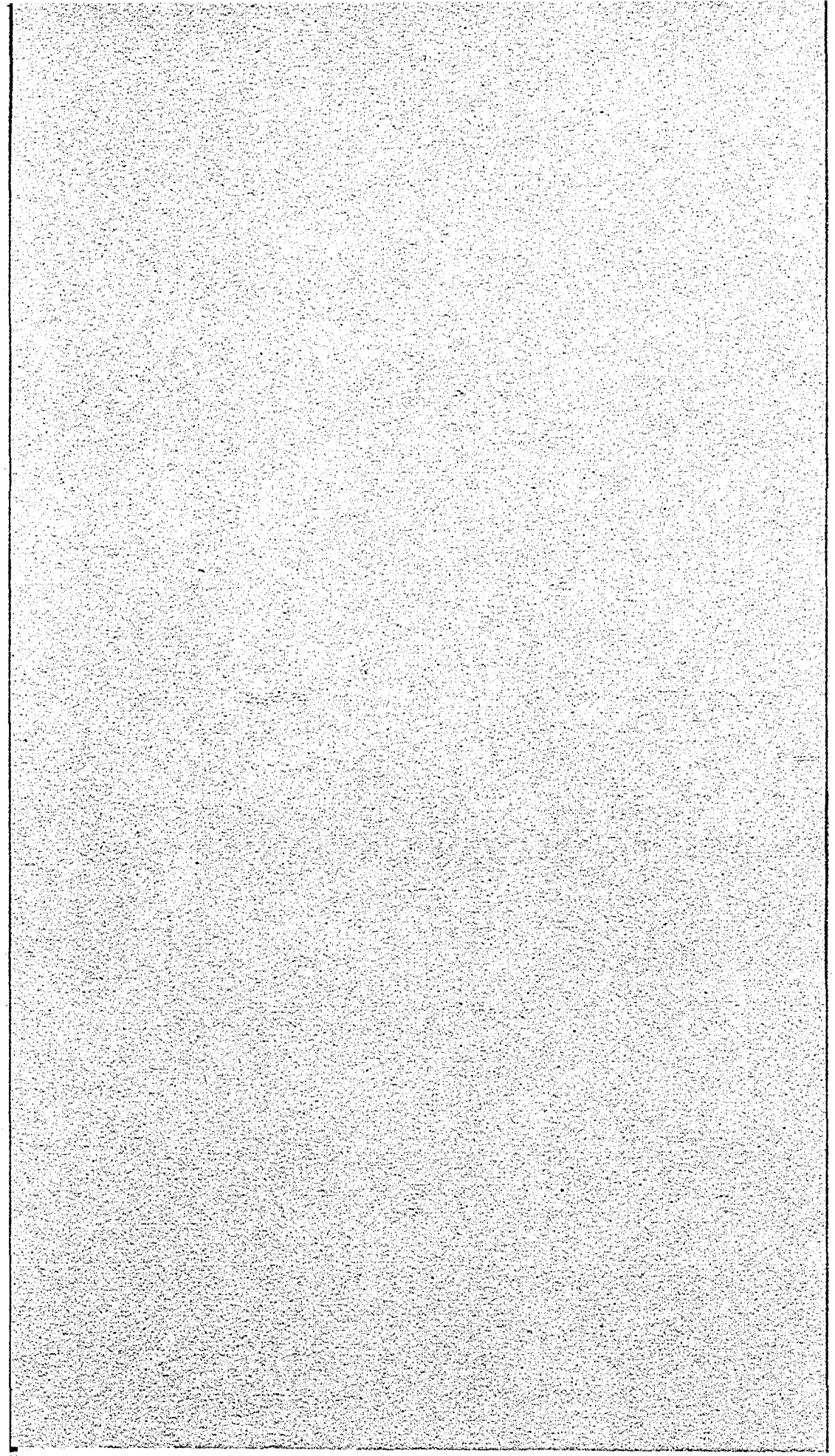

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ORGANIC MATTER AND THE GROWTH OF LEMNA

INTRODUCTION AND HISTORICAL

For about seventeen years it has been known that all animals require in their diet minute quantities of organic substances of unknown composition called vitamins, in order to grow and live normally. These accessory substances have not as yet been shown to be non-essential to the life of animals, but seem to be necessary for their proper nutrition.

Wildiers (45) advocated the necessity of some organic substance for the normal growth and reproduction of yeast. He named this accessory yeast factor, "bios". Some investigators supported his view, but many believed that yeast could grow quite well in a medium containing no "bios". There was, however, little progress made toward settling this question until recently, when Fulmer (23), Nelson and McCollum and his co-workers (27), showed that this "bios" of Wildiers, is an accelerator rather than an essential, and can not be compared to animal vitamins. This substance, then, which was considered by many investigators in the field of nutrition to be essential to satisfactory yeast growth, and which has recently been shown to be non-essential, is a yeast accelerator.

The possibility of the presence in the soil of growth-promoting accessory materials was advocated by Bottomley (6, 7, 8, 10, 11) and by Mockeridge (30, 31, 33). They stated

that the green plant can not grow and reproduce normally if minute quantities of certain organic substances were not present. These organic materials which were believed to be analogous in function to vitamins in animal nutrition were called by Bottomley, "auximones". "It is now established that plants, in their turn, require growth-promoting substances, or auximones, which in the case of the lower plants, are apparently manufactured by themselves, but which in the case of green plants must be supplied from without. Since these necessary accessory substances are essentially organic in nature, their only possible source in the case of ordinary green plants is to be found in the organic matter in which they are growing".

Bottomley employed Lemna major and Lemna minor, both of which reproduce by budding, and whose use avoids introducing the auximone in the seed. These plants were inoculated into Detmer-Moor's and Knop's Solutions, and after three weeks it was noticed that their weight and appearance were appreciably below normal. When small quantities of organic matter, extracted from soil and "bacterized" peat, were added to these inorganic salt solutions, the rate of reproduction and health of the plants soon began to increase. Mockeridge obtained similar results with Lemna major using Detmer's and Knop's Solutions.

Bottomley, then, after failing to obtain satisfactory growth with Lemna in these inorganic solutions, seems to have

tried no other, but concluded that the green plant could not grow and reproduce satisfactorily without the presence of auximones. While Breazeale, (12) Chittenden (14) and Rosenheim (35) did not make an exhaustive study of this question, they were lead to believe that vitamin-like substances were necessary for the good growth and healthy appearance of green plants. Mendiola (29), however, in a genetic study of Lemna major, reported good growth, and even an increased size of plants with a modified Pfeffer's Solution which contained no organic matter. Similar results were obtained by Wolfe (47) in 1927 while working with Lemna minor. He employed several three-salt solutions advocated by the National Research Council, and with some of these he secured good growth over a period of thirty-six days, but failed to get any increase in reproduction by the addition of small amounts of various pure organic substances.

Lumiere (26), after working with various fungi and Lemna minor in inorganic and organic media, concluded that both lower and higher plant life could grow normally in media containing no organic matter, and that accessory substances were not essential. He noted, however, that when organic matter stimulated plant reproduction, this stimulation was due to products formed either from vitamins, or from modified vitamins present in the organic substance and was not caused by the direct action of any accessory material.

In 1924, Clark and the writer[⊠] (18), while working under

⊠This part of the thesis was published jointly with Dr. Norman A. Clark, and a reprint is inserted for convenience.

the direction of the former, used the three salts-monocalcium phosphate, potassium nitrate, and magnesium sulfate, with iron supplied as ferric phosphate, as the basis for media in which to grow Lemna major. Various concentrations of these three salts were made up, and those in which the plants grew and reproduced normally were determined. One solution was finally chosen as satisfactory for Lemna and was employed for later work. In this inorganic medium the plants have grown satisfactorily for several years. Some slight changes, however, have been made in the composition of the solution, and the plants have continued to reproduce and to appear as healthy as those grown under natural conditions.

Since these green plants have maintained their health and reproduction rate while growing in a solution made from recrystallized salts and three-time distilled water containing no organic matter, auximones can not be regarded as essentials for the growth of Lemna in the same way as vitamins are essentials for the growth of animals. Their function, therefore, is that of an accelerator, just as is bios in connection with the growth of yeast.

Saeger (37) confirmed the results of Clark and Roller by growing Lemna major in a diluted Knop's Solution without adding organic matter, but he suggested that auximones might have been added as impurities in the salts he used or possibly supplied by microörganisms present in his solutions.

The results of Clark and Roller were again confirmed by

Ashby (3) in 1928 at the University of London. He grew Lemna minor in their solution for six months without the addition of organic matter and during that time it remained healthy and reproduced normally. For this work Ashby (1 and 2) used a growth chamber with light, temperature, nutrition and aeration held constant. He does not state, however, whether or not he crystallized his salts used.

Since the possibility that green plants need organic substances as essentials has been removed, the question of stimulation of growth and reproduction by organic matter constitutes a problem which will be considered later in this thesis.

METHODS AND TECHNIC

In this work Spirodela polyrhiza (L.) Schleiden, or Lemna major, was used. According to Britton (13), this plant belongs to the Lemnaceae or greater duckweed family and reproduces by budding. Its thalli or fronds, ranging from 2 to 10 m.m. in length, are thick flat and dark green above, but slightly convex and purple beneath, and are palmately nerved, bearing a central cluster of from 4 to 16 elongated roots which contain root-caps. Several Lemna were obtained from the reserve stock maintained by Clark and Roller in their inorganic nutrient medium. These plants had been growing in this solution for several years and were looking healthy. The solution employed in this investigation is the one described by Clark and Roller (18) in their paper on "Auximones and the Growth of the Green Plant", with the exception of the source of iron.

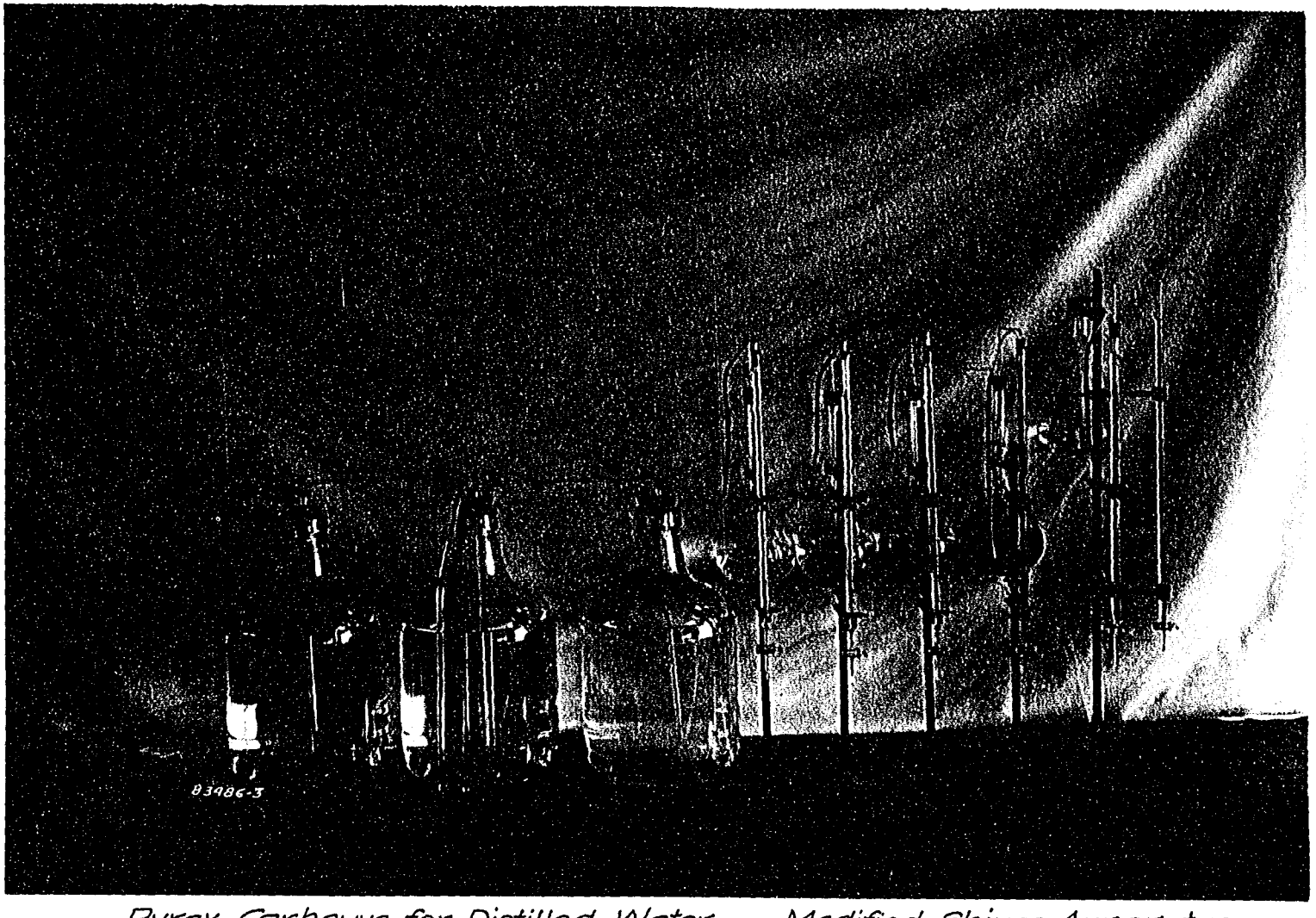
Ferric chloride was used instead of ferric phosphate. The solution used at this point of the investigation has the following composition:

TABLE I. SOLUTION NO. 1

Salts	Composition of Stock Solution: cc. of stock sdn used per 250 cc. nutn. soln.	Crystalizations	Gms. in 2 liters	Gms. in 1 cc.	Mols per Liter	Mols per cc.	Grams of element per cc.	Composition of nutrient solution: Mols of element per liter	Grams of element per liter
$CaH_4(PO_4)_2.H_2O$	2	2	25.22	0.0126	0.05	0.00005	0.002	0.0004	0.016
KNO_3	5	3	80.888	0.0404	0.04	0.00004	0.0156	0.008	0.312
$MgSO_4.7H_2O$	5	3	24.649	0.0123	0.05	0.00005	0.0012	0.001	0.024
$FeCl_3.6H_2O$	5	2	0.3000	0.00015	--	--	0.000031	--	0.000062

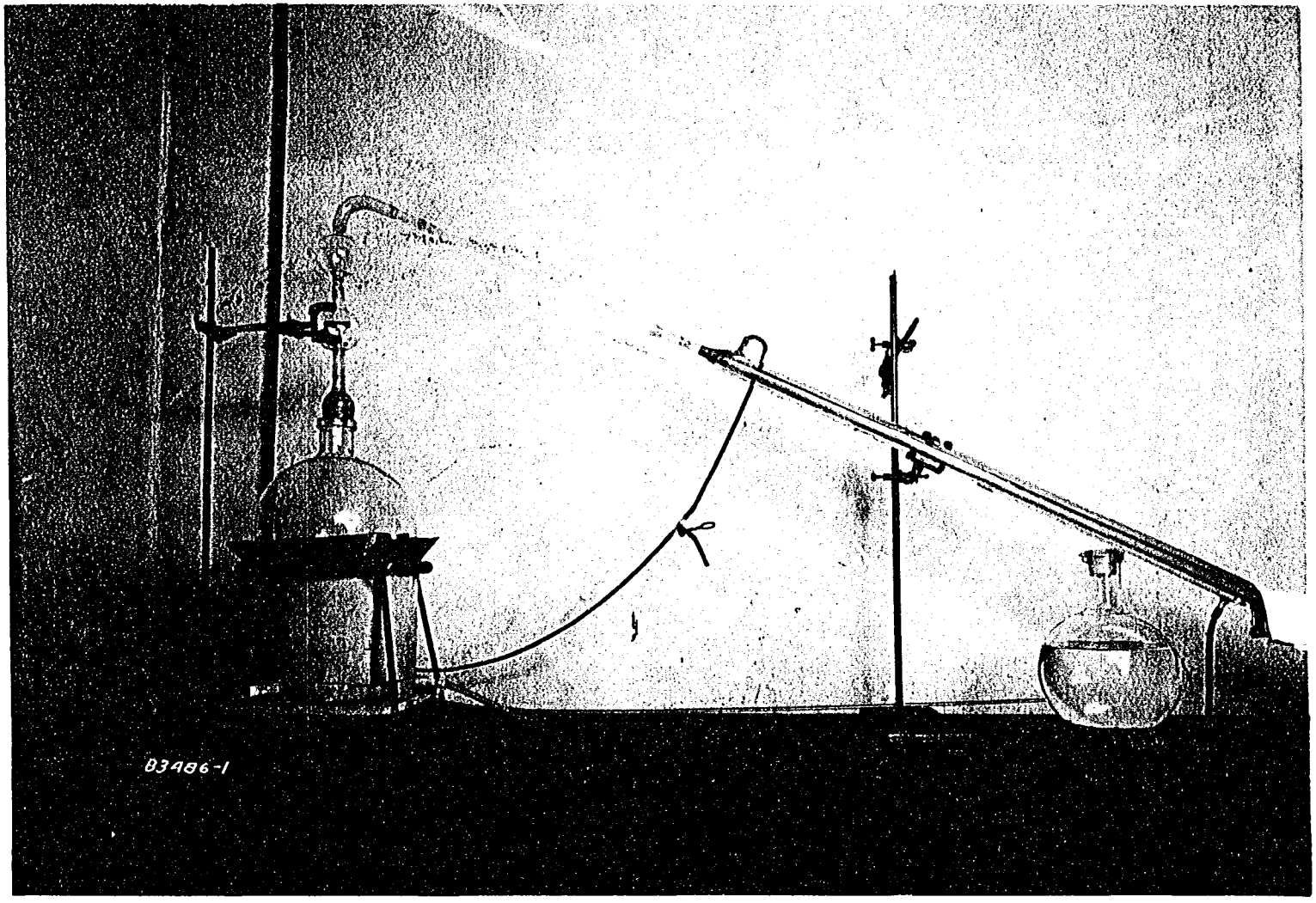
All of the four stock solutions whose compositions are given in Table I were kept in the modified Shive's apparatus (44) of pyrex shown in Figure 1. In the last column of the table are listed the number of cubic centimeters to be measured out for each 250 c.c. of the nutrient medium, the volume used for growing Lemna. The water employed had been distilled three times--the second distillation being made with alkaline potassium permanganate and the last one through pyrex glass. This still is shown in Figure 2. The water used, therefore, for nutrient solution work contained only those metals present in pyrex glass. It was stored in the large uniform carboys shown in Figure 1. We considered it advisable, however, to allow this water to stand in these carboys not longer than two weeks for fear that it would dissolve some of the glass, or gases from the air, either one of which might offer a source of injury to the plants.

The technic followed in this nutrient solution work is comparatively simple. 250 c.c. of the nutrient solution were then made up according to Table I and poured into 250 cc. pyrex beakers, and covered with petri dish lids in order to keep dust out.



Pyrex Carbouys for Distilled Water Modified Shives Apparatus

Figure 1.



Pyrex Still

Figure 2

Ten plants were usually employed for inoculation. These were changed to fresh solutions twice a week and when they had increased to sixty they were cut down to about twenty-five, care being taken to leave the best each time. All plants were grown at a temperature of 25°C. in a thermostat which was placed in a south window in order to receive the greatest amount of sunshine. (See Figure 3 for illustration)

Clark (15) in his paper entitled, "The Rate of Reproduction of Lemna Major as a Function of Intensity and Duration of Light" reports the rate of reproduction as being directly proportional to the time of illumination. He used Mazda lamps with average intensities of 400 and 900 foot candles at the surface of the solution, and at both intensities he gradually increased the length of exposures from 12 hours to 24 hours. At both intensities the greater the exposure the greater the rate of reproduction. The 900 foot candle, however, at any length of exposure gave a greater rate than the 400. It seems, therefore, that light is an important factor in the growth of this plant, and to maintain constant growth and reproduction the supply of this factor must be constant. In an attempt to furnish a constant supply of light, rather than a maximum amount a south window was chosen. A double layer of cheese cloth was utilized to cover the window on clear days in order to avoid excessive heat from the direct sunshine. Otherwise the temperature would rise above 25°C. and this would necessitate too much cooling of the thermostat.

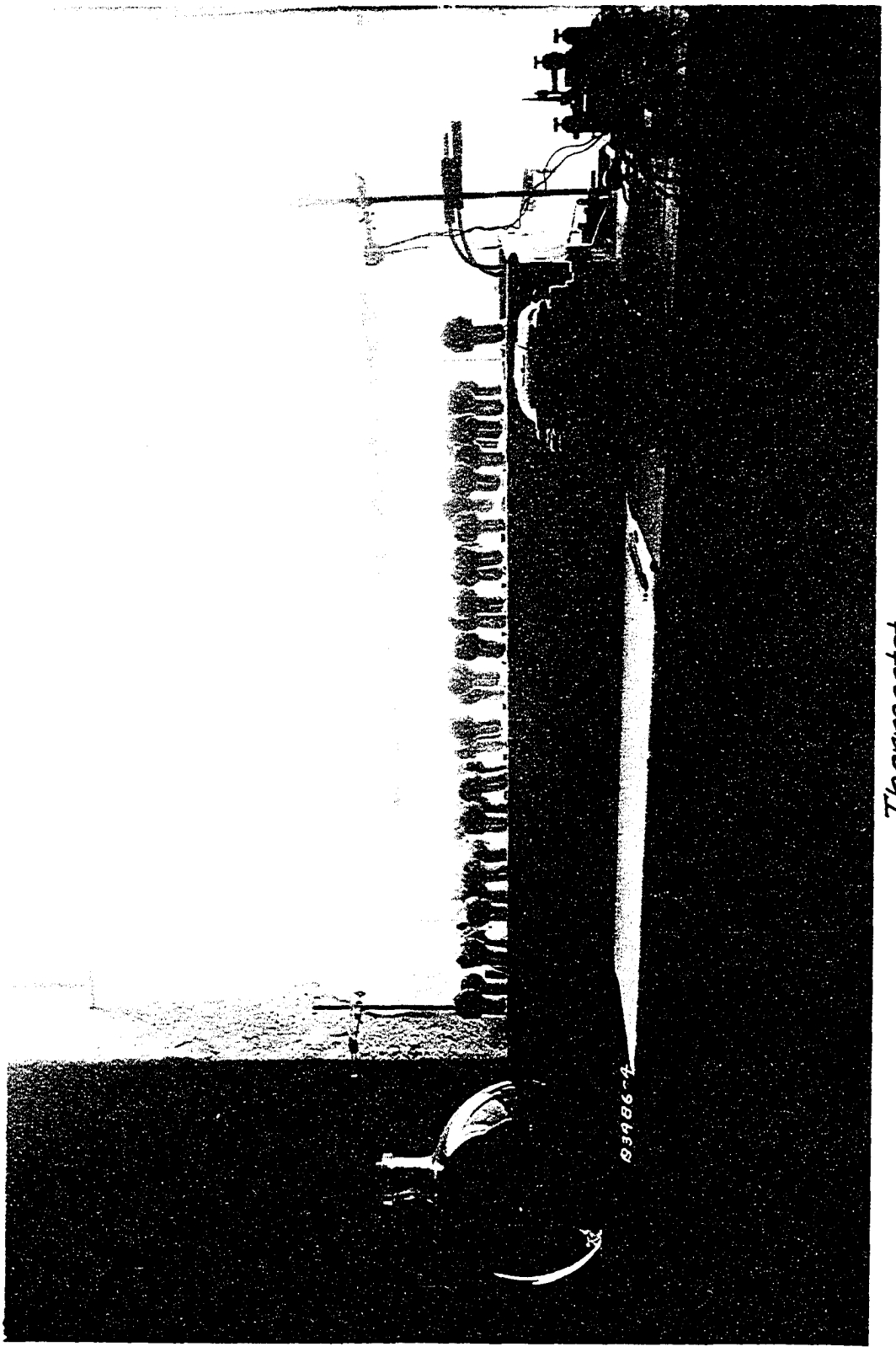


Figure 3.

Usually a count of the plants was taken every three days, at which time observations were made on length of roots, size and color of fronds, and general appearance. Given favorable conditions, the rate of increase at any time should be directly proportional to the number of plants present, whence,

$$(1)\quad \frac{dn}{dt} \propto n$$

Introducing constant k (1) becomes

$$(2)\quad \frac{dn}{dt} = kn$$

or

$$(3)\quad \frac{dn}{n} = k\,dt$$

Integrating between limits (3) becomes

$$(4)\quad \int_{n_o}^{n} \frac{dn}{n} = k \int_{t_o}^{t} dt$$

or

$$(5)\quad (\ln n)_{n_o}^{n} = k\,(t)_{t_o}^{t}$$

or

$$(6)\quad \ln n - \ln n_o = k\,(t-t_o)$$

or

$$(7)\quad \ln \frac{n}{n_o} = k\,(t-t_o)$$

As a result of these computations equation No. 7 is that used to determine the slope of the growth rate curve. This curve with slope k is obtained by plotting the logarithm of the number of plants against time. The variation in temperature and sunlight, and possibly the time of the growing season would be expected to make the curve somewhat irregular.

For convenience it is further shown how the number of generations and the generation time of Lemna can be derived.

Let n = final number of plants at time (t).

Let n_o = initial number of plants at time (t_o).

Let x = generation time for one frond.

Let g = number of generations for time (T).

The final number of plants which should follow the organic rate law are equal to the initial number multiplied by two which has been raised to a power equal to the number of generations less one, whence

(1) $n = n_o\ (2^{g-1})$

(2) $2^{g-1} = \frac{n}{n_o}$

(3) $(g-1)\ \ln 2 = \ln \frac{n}{n_o}$

(4) $\ln 2^g - \ln 2 = \ln \frac{n}{n_o}$

(5) $\ln \frac{2^g}{2} = \ln \frac{n}{n_o}$

(6) $\frac{2^g}{2} = \frac{n}{n_o}$

(7) $2^g = \frac{2n}{n_o}$

(8) $g \ln 2 = \ln \frac{2n}{n_o}$

(9) $0.301g = \ln \frac{2n}{n_o}$

(10) $g = \frac{\ln \frac{2n}{n_o}}{0.301}$ = number of generations

Since $\frac{g}{T} = x$, or generation time, (10) becomes

(11) $\frac{\ln \frac{2n}{n_o}}{0.301} \times \frac{1}{T} = x$, or generation time

Using this modified solution of Clark and Roller (18) and with the methods and technic just given, we attempted to grow

Lemna, but with very little success. After some preliminary experiments with the addition of hydrochloric acid and potassium hydroxide, the difficulty was found to be in the reaction of the nutrient medium, for when a small quantity of a 0.05 normal solution of KOH was added, the plants soon began to look better and to grow faster. This suggested, then, the work which was later done on the optimum pH of nutrient media.

Clark (16) showed that the best growth was obtained for Lemna major when the plant was grown in solution 1, whose pH had been adjusted to 4.8. The writer using the same nutrient medium checked the results of Clark. Ashby (3) later confirmed our optimum pH value of solution 1.

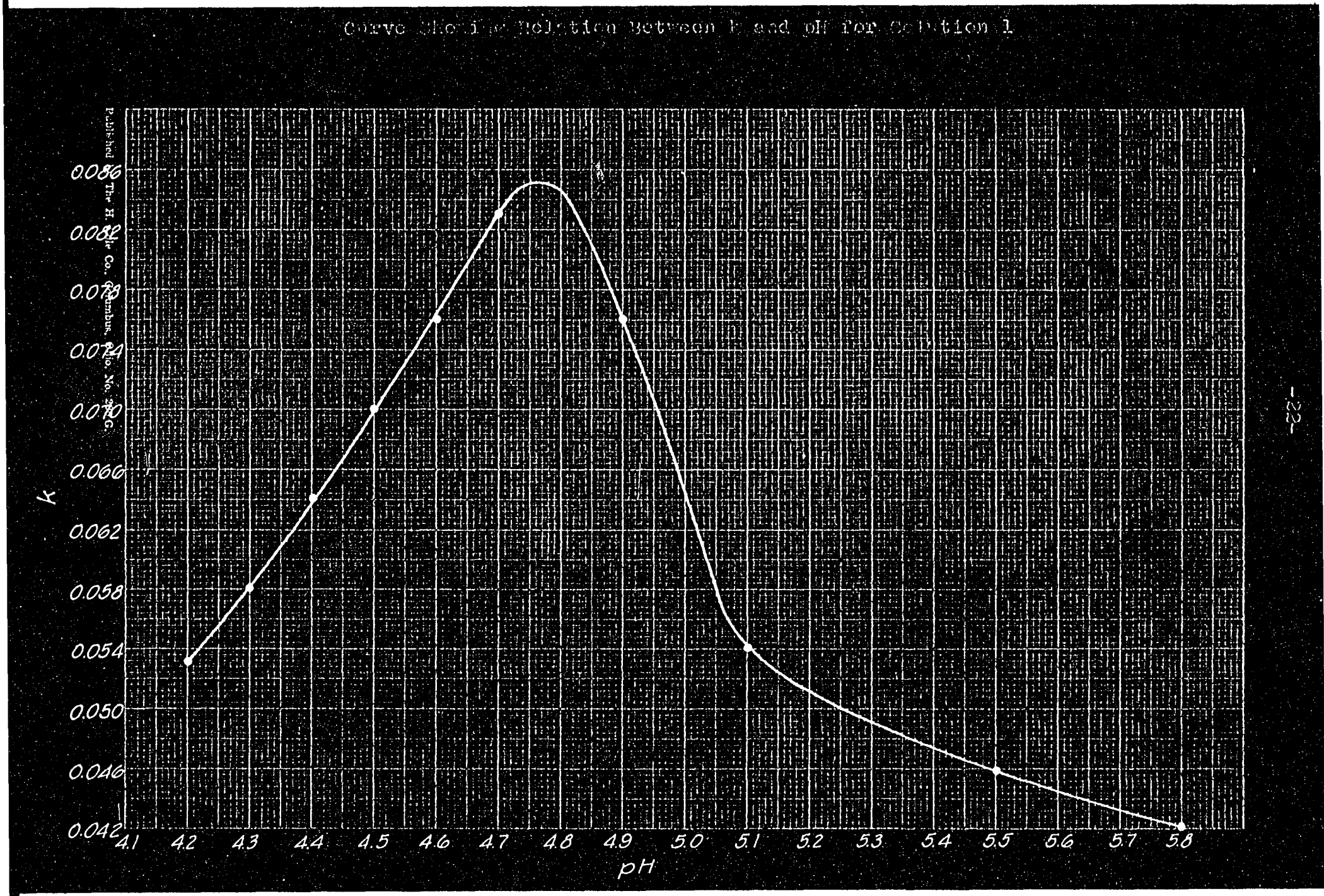
The LaMotte apparatus was at first employed in this investigation in order to determine the hydrogen ion concentration of the various solutions, and to adjust solutions to definite pH values. Later, however, a potentiometric set-up in which the quinhydrone electrode as described by Collins (19) was employed instead of the hydrogen electrode.

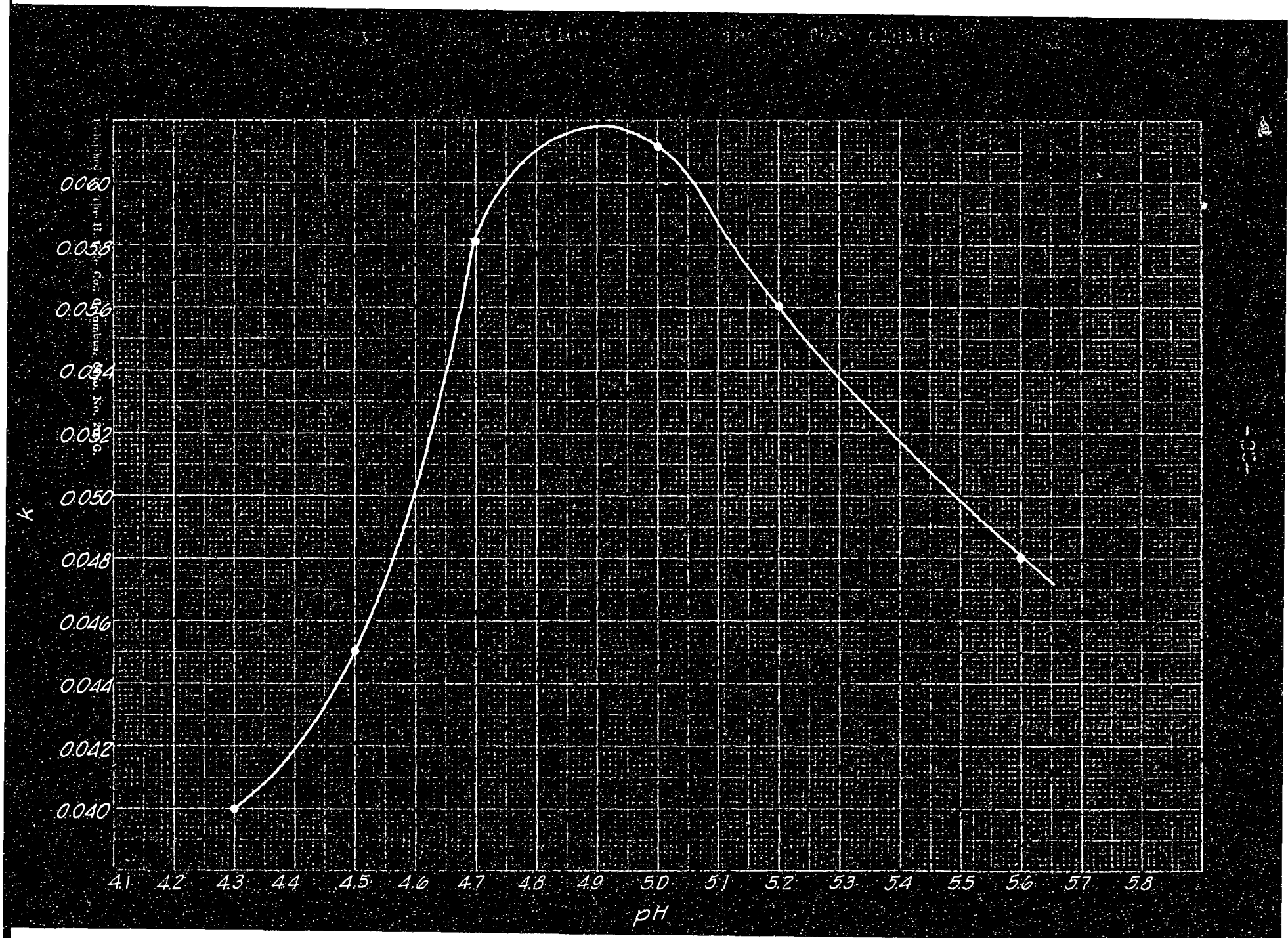
More satisfactory results were obtained with the apparatus when certain precautions were taken. Before using, we allowed a few drops of the saturated KCl solution to run out of the electrode, then sealed the top with paraffin. Not a single drop of saturated KCl should flow into the solution whose pH is to be determined. Before using, the platinum electrode should be cleaned at least three times by heating it in an alcohol flame, dipping into freshly prepared cleaning

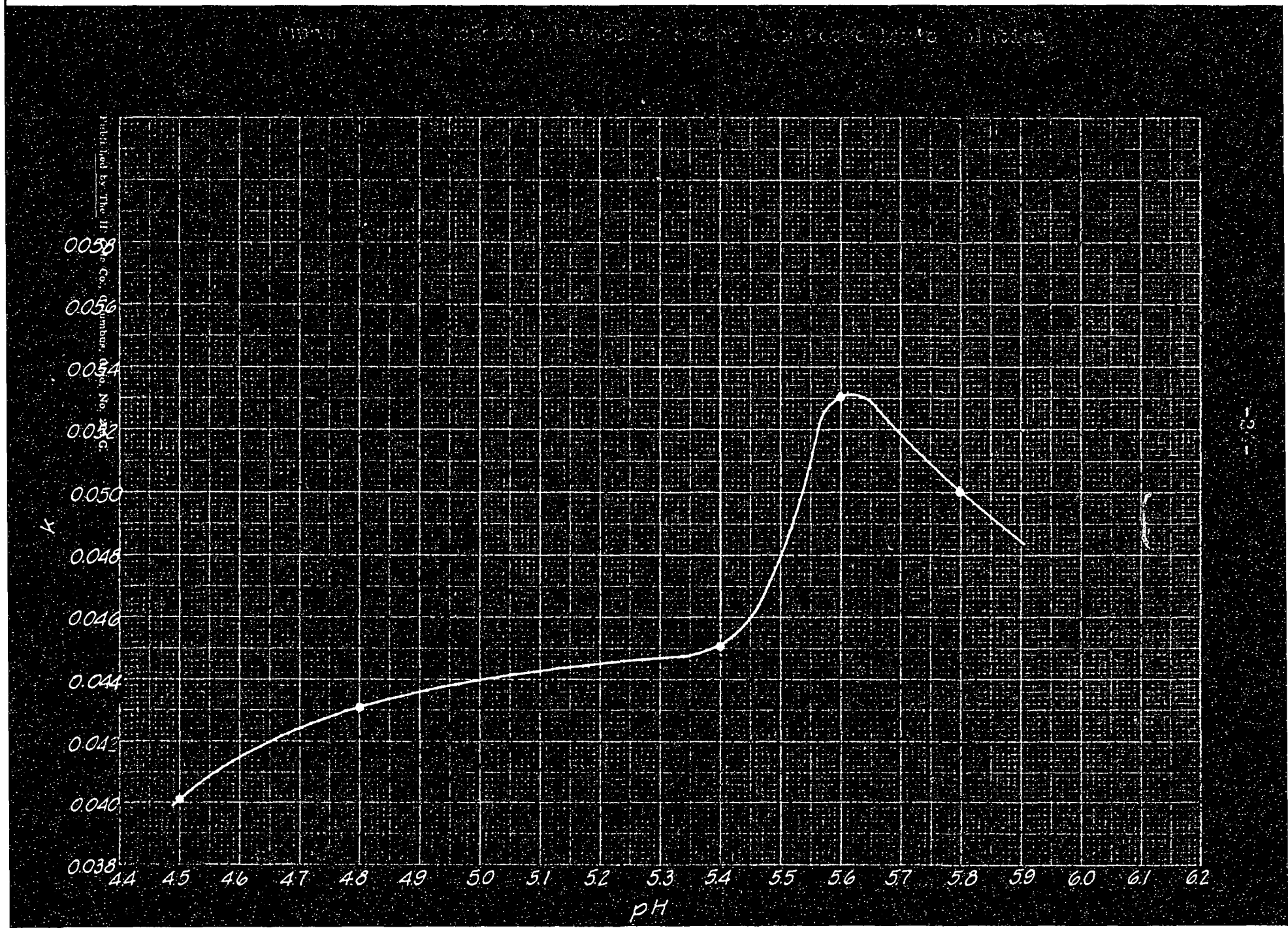
solution and then washing clean with distilled water. While not in use, the two electrodes should be immersed in a saturated solution of KCl. When determining the pH of a solution, neither electrode should touch the side of the container, nor should the glass of the platinum electrode extend into the solution. The electrodes must not be agitated at any time.

A quantity of solution 1 was prepared and divided into three parts. Each portion was given to separate individuals, who determined the pH values by using simultaneously their quinhydrone electrode and set up at the same temperature. The difference between the extreme values which these individuals obtained was not greater than a pH of 0.1, a result which checks the LaMotte apparatus very closely. The accuracy of the quinhydrone electrode in a like manner has been checked several times subsequently. Each time before using, its accuracy was tested against a standard solution of $\frac{m}{20}$ potassium acid phthalate whose pH is 3.97.

The following table and graphs attempt to show the effects of change in the concentration of a nutrient medium on the optimum pH, and the relative growth rates of Lemna in the various solutions at their optimum hydrogen-ion concentration.


Curve showing relation between k and pH for Solution 1
Published The H. [illegible] Co. Columbus, O. No. [illegible] G.
k
0.086
0.082
0.078
0.074
0.070
0.066
0.062
0.058
0.054
0.050
0.046
0.042
4.1
4.2
4.3
4.4
4.5
4.6
4.7
4.8
4.9
5.0
5.1
5.2
5.3
5.4
5.5
5.6
5.7
5.8
pH


0.060
0.058
0.056
0.054
0.052
0.050
0.048
0.046
0.044
0.042
0.040
k
4.1
4.2
4.3
4.4
4.5
4.6
4.7
4.8
4.9
5.0
5.1
5.2
5.3
5.4
5.5
5.6
5.7
5.8
pH


0.058
0.056
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0.052
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0.046
0.044
0.042
0.040
0.038
K
4.4 4.5 4.6 4.7 4.8 4.9 5.0 5.1 5.2 5.3 5.4 5.5 5.6 5.7 5.8 5.9 6.0 6.1 6.2
pH

Curve Showing Relation Between k and pH for Saeger's Solution

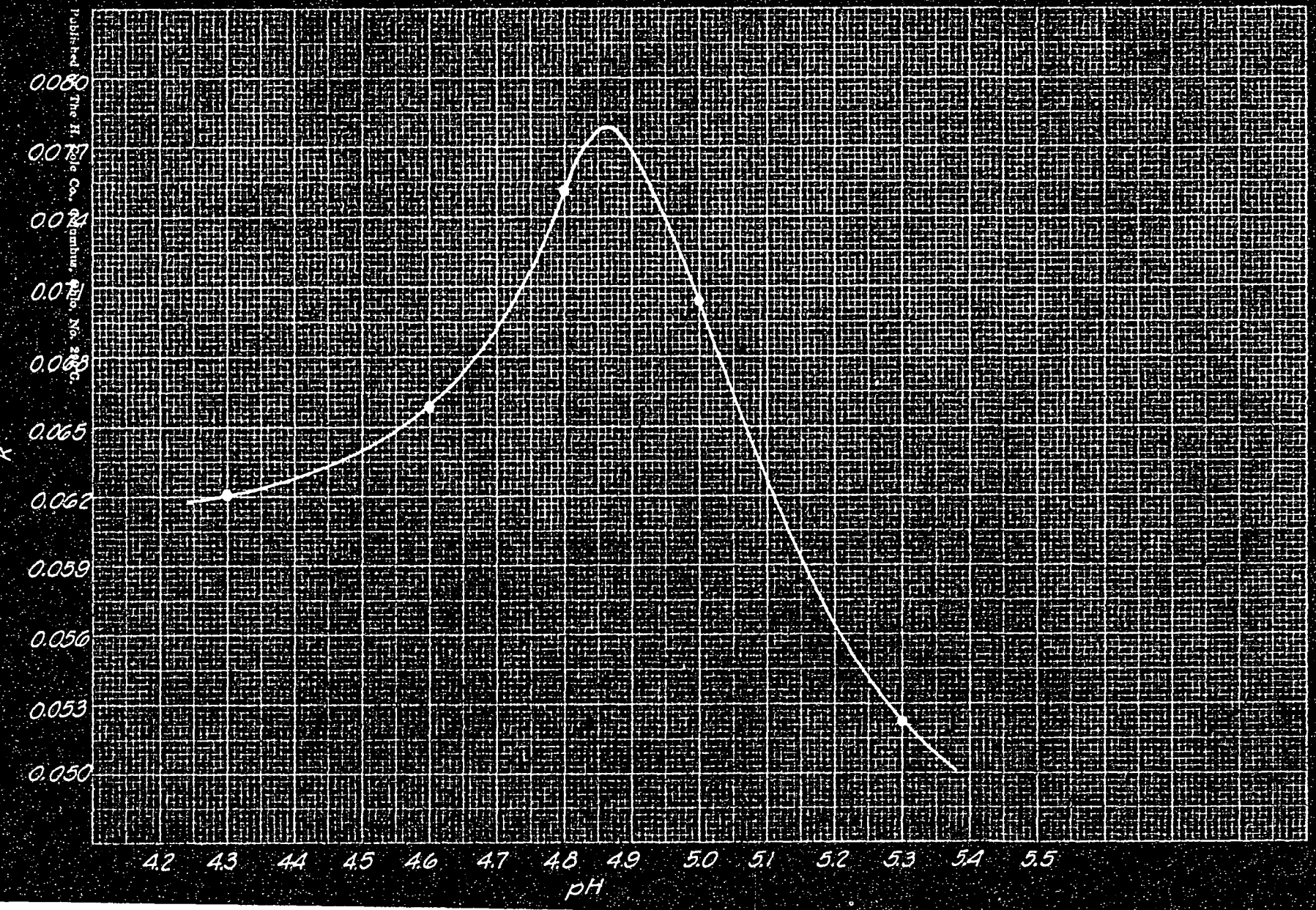


TABLE II. CONCENTRATION IN MILLIGRAMS PER LITER OF SEVERAL NUTRIENT SOLUTIONS.

	Solution 1	Solution 2	Solution 3	Solution 4
	Clark's 1st modification of Clark and Roller's Solutions	Clark's 2nd modification of Clark and Roller's Solutions	Bottomley's modification of Knop's Solution	Saeger's modification of Bottomley's Solution
K	312	19	112.4	112.4
Mg	24	72	16.5	16.5
Ca	16	40	28.2	28.2
Fe	0.6	0.6	few drops of $FeCl_3$ soln.	8.3(0.56 remains in solution
pH	4.3	4.7	4.7	3.6
Opt. pH	4.8	4.9	5.6	4.9
k at pH	0.053	0.058	0.040	plants die
k at Opt. pH	0.086	0.062	0.053	0.078

A more detailed composition of the solutions mentioned in Table II is given below:

Solution 1: A detailed composition is given in Table I.

Solution 2: A detailed composition is given in Table III.

TABLE III.

Salts	Composition of Stock Solutions: c.c. of stock sol. used per 250 c.c. of nutr. sol.	Crystallizations	Gms. in 2 liters	Gms. in 1 cc.	Mols per liter	Mols per c.c.	Gms. of element per c.c.	Composition of Nutrient Solution: Mols of element per liter	Grams of element per liter
KH_2PO_4	0.6	2	54.46	0.0272	0.2	0.0002	0.008	0.00048	0.019
$Ca(NO_3)_2 4H_2O$	5	2	23.616	0.0118	0.05	0.00005	0.002	0.001	0.040
$MgSO_4.7H_2O$	15	3	24.649	0.0123	0.05	0.00005	0.0012	0.003	0.072
$FeCl_3.6H_2O$	5	2	0.300	0.00015	0.0006	--	0.000031	--	0.00062

Solution 3

KNO_3..... 1 gram
KH_2PO_4..... 1 gram
$MgSO_4.7H_2O$..... 1 gram
$Ca(NO_3)_2.4H_2O$..... 3 grams
Few drops of $FeCl_3$ solution
Distilled water..... 6 liters

Solution 4

KNO_3	1 gram
KH_2PO_4	1 gram
$MgSO_4.7H_2O$	1 gram
$Ca(NO_3)_2.4H_2O$	3 grams
$FeCl_3.6H_2O$	0.24 grams
Distilled water	6 liters

The results listed in Table II show that the optimum pH for the growth of Lemna in solutions 1, 2 and 3 are significantly different, while in solution 4 it is the same as the optimum in solution 2. One notes that the growth rate constants at the optimum pH of each solution differ, but this may be due to the fact that these constants were obtained at different times when the light intensities were probably not the same. The compositions of the various solutions in Table II and the fact that Lemna grew well in each solution at its optimum pH seem to indicate that the calcium-magnesium ratio is less important than the hydrogen-ion concentration, that the concentration may be varied over a moderate range as long as the hydrogen-ion concentration is maintained at the optimum for each change in concentration, that the concentration of potassium may be varied within wide limits, and that the calcium-magnesium ratio may be reversed, as it is in solutions 1 and 4, as long as the pH is held at the optimum in each case, and good growth can be obtained.

Saeger (37) attempted to grow Lemna in his modification of Bottomley's (11) Solution without satisfactory results, but he obtained fair growth when this solution was diluted

1:10 and 1:50. He states that the reason for this is unknown. Using only one dilution, 1:50, we confirmed Saeger's results. From Table II, it is seen that the pH of Saeger's solution is 3.6 which is appreciably below the optimum, 4.9. When this solution was diluted 1:50 the pH was changed to 5.1 which is only slightly above its optimum. It seems, therefore, that the reason for the plant not growing in the undiluted solution, though growing in the diluted, can be attributed to the hydrogen-ion concentration of the two media. It is probable that Bottomley and Mockeridge could have grown Lemna more successfully in Knop's and Detmer's Solutions had they adjusted the pH of these to their optimum. Wolfe (47) considers that there is now sufficient evidence to show that the failure of Bottomley to grow plants successfully in purely inorganic solutions was due to the lack of physiologically balanced media. This failure may be caused by an unfavorable pH and not by unbalanced nutrients.

HCl and KOH were used to adjust the pH of the various solutions. It was found that the chloride and potassium ions added in the above compounds had no significant effect on the growth-rate of the plants. Similar results were obtained with the nitrate ion.

In the summer of 1928, it was noticed that manganese in the form of $MnCl_2$, when added to solution 1 in the concentration of 1:1000000 stimulated the formation of chlorophyll, and increased the size of the plant. Mr. Fly, while working later in this laboratory, found the optimum concentration of

this element for solution 1 to be about 1:10,000,000 for the growth of Lemna major. The manganese used by him was from recrystallized $MnCl_2$. This stimulant was used then as a part of solution 1 for later work.

Solution 1, whose composition is found in Table I, served quite satisfactorily as a medium for the growth of Lemna when the pH was adjusted to 4.8, and 1:10,000,000 of manganese was added. This medium, then, with the pH and manganese modifications will be used for nearly all nutrient solution work described later in this thesis, and will be designated as modified solution 1.

EXPERIMENTAL

Since the possibility that auximones present in organic matter are essential for the satisfactory growth and reproduction of green plants had been removed by the work of Clark and Roller (18) we attempted to show what effects organic matter had on the green plant, using the methods and technic already given. This work was accomplished in part with solution 1 without the pH or manganese modifications, and with one and both adjustments. The results of these experiments will be given later in this thesis.

In July, 1925, while growing Lemna in solution 1 to which had been added aqueous extracts of yeast, carrots, alfalfa, barley, soil and manure, we noticed a heavy growth on the plant roots in all solutions excepting the inorganic nutrient medium and those containing soil and manure extracts. This growth appeared to be a fungus contamination which was so firmly attached to the roots that any attempt to wash it entirely free was unsuccessful. In July, 1926, a similar observation was made while growing Lemna in solution 1, with pH modification, containing urea and asparagin; in July, 1928, with alfalfa aqueous extract; and again in September, 1928, with four fractions of alfalfa aqueous extract, made according to Fulmer, Duecker and Nelson (22).

A microscopic examination of the growth on some of the roots revealed the presence of large numbers of fungi, algae, bacteria and protozoa. The same classes of micro-organisms

were also found on and in the roots and on and in the frond of the green plant which had been growing in the inorganic medium, but not in such abundance. We see, then, that all the various organic substances tried, except soil and manure aqueous extracts, stimulated the growth of these organisms and consequently offered difficulties in making accurate observations on plants grown in solutions containing such organic materials.

Mockeridge (33) in 1924 reported in a paper "The Formation of Plant Growth-Promoting Substances by Micro-Organisms", that nucleic acid derivatives increase the growth of Lemna minor, as do autolyzed yeast, a crude extract of peat and sterilized cultures of Azotobacter. In fact, according to her, all organic substances which have been found to stimulate the growth of the green plant in minute quantities contain these purine and pyrimidine bases or nucleic acid derivatives; so it would seem that auximones or plant growth-promoting substances can be furnished the growing plant in a strictly inorganic medium by Azotobacter and yeast inasmuch as she showed them to contain these derivatives. The suggestion was made that some of the auximones may be direct products of bacterial metabolism. This suggestion was also made by Saeger (37) after successfully growing Spirodela polyrhiza in a diluted Knop's Solution containing no organic matter.

Since the technic used by Clark and Roller (18) in the

purification of salts, the distillation of water, the care of glass ware and the changing of plants was followed in our work, the most likely possibility for any organic substance of getting into the nutrient medium is the micro-organisms already present in the solutions. Then, in order to show that the green plant does not depend upon micro-organisms to furnish organic substances, it will be necessary, first, to sterilize the plant and then to grow it in a sterile inorganic solution.

ATTEMPTS TO STERILIZE LEMNA

The object, therefore, of freeing Lemna major of all micro-organisms is three-fold, namely: to eliminate all micro-organic growth which contaminates the roots and consequently offers great difficulties in making accurate observations on the plants grown in some organic solutions; to show that Lemna does not depend upon bacteria to furnish organic materials for its proper nutrition; and to show what effects sterile organic substances have upon the growing sterile plant.

Examination of the literature reveals that Hansteen (25) in 1899 attempted to sterilize Lemna minor by repeatedly washing it with sterile water. After this operation it was transferred to a flask containing sterilized egg albumen, and incubated for several days. Hansteen's criterion of sterility was the development of a turbidity in the solution and the subsequent evolution of ammonia, otherwise the flasks and their contents were considered sterile. No mention is made

in his paper about the length of time necessary to wash the plant free of microorganisms, nor are there any details of his technic given.

This work was repeated by the writer. Twenty single fronds of Lemna major were washed with sterile water for one hour and transferred to separate flasks containing sterile egg albumen. After these solutions had been incubated for several days, there was observed a turbidity in some but none in others, and in some of the turbid solutions the odor of ammonia was in evidence. A small portion from each flask was then transferred by means of sterilized pipettes to bacto-nutrient medium, and after several days bacterial growth was noticed on all the tubes containing the agar medium. This shows that none of the twenty original fronds had been sterilized by Hansteen's method.

An attempt was now made to free Lemna of microorganisms by means of ultraviolet light. For this purpose a Hanovia mercuric vapor lamp, described by Ellis and Wells (21) was used. When agar plates, which had been exposed to the air in the laboratory for five minutes, were held five inches from this lamp all micro-organisms were destroyed in thirty seconds.

Single fronds were washed well with modified solution 1, were transferred to a transparent quartz Erlenmeyer flask of 50 c.c. capacity containing enough of the above solution to float the plants, and were exposed equally on top and bottom five inches from the quartz mercury vapor lamp. To prevent

the flask becoming too warm, it was not held directly over the top but to one side of the lamp. Using this technic, we exposed plants to ultraviolet light for lengths of time, varying from one-half minute to one hour, and then transferred them to sterile bacto-nutrient agar. After several days incubation there was noticed bacterial growth on all plates. Several plants were exposed again in a like manner, and transferred to a sterile nutrient medium whose composition was solution 1 with manganese and 1 gram of solid bacto-nutrient agar per liter of medium. Bacterial growth developed in all flasks after a few days. Plants whose exposure had been longer than six minutes died. Others that had been subjected to less than six minutes were harmed but lived. While the light killed many micro-organisms, it did not kill all of them. Alternate shaking and exposure to the lamp not longer than six minutes did not kill all bacteria, but it helped to do so. It was further shown that single fronds, when exposed to ultraviolet light from one to five minutes on three successive times, and when kept in sterile nutrient solution 1 between the successive exposures were not sterilized. Sufficient time passed during these exposures for the second and third generation buds to form. The buds even produced bacterial growth on bacto-nutrient agar. (All solutions were sterilized by autoclaving under 20 pounds pressure for 15 minutes while soil and other suspensions were subjected to two such treatments. Three days were allowed for the development

of spores before the final autoclaving. In all cases tested, this treatment was sufficient for complete sterilization.)

Several plants and roots which were examined with the oil immersion objective revealed the presence of bacteria in the interior of the root and frond. In many cases this seemed to be verified by the fact that when some fronds were exposed to ultraviolet light and dragged on both sides over the surface of the bacto-nutrient agar, bacterial growth developed only around the plant.

A centrifuge operated at 4500 r.p.m. with the plant suspended in sterile solution 1 on a gauze had scarcely more sterilizing effect than ultraviolet light, even after one hour of centrifuging. One minute exposure to the lamp and centrifuging for ten minutes alternately for two successive times, allowing three days to intervene between each treatment did not produce sterile Lemna; nor did freezing the plant in solution 1 and exposing it to ultraviolet for four minutes produce the desired result. Again some plants were put into a nutrient medium and were kept at 0°C. for several days but were changed to fresh solutions twice a week during this period. After this they were exposed to ultraviolet light for four minutes, and then centrifuged for 5 minutes. This process was continued for several weeks, and after each exposure to the light, one single plant was plated out, but the number of micro-organisms did not seem to decrease with each operation.

Winter buds were produced from Lemna by adding a few drops of a saturated solution of $Ca(OH)_2$ to the nutrient medium in which they were growing, by adding a drop or two of dilute HNO_3, or by transferring the plants to distilled water and adding $Ca(OH)_2$ as before. These may be produced in fact by growing the plants under abnormal solution conditions. Winter buds were obtained and subjected to the action of ultraviolet light for varying lengths of time without any more success than was secured with the normal plant. Some of these buds as well as normal fronds were exposed in an evacuated transparent quartz tube to the lamp, but without success.

Several organic and inorganic substances with germicidal properties were next tried out. By following the report of Wilson (46) they were separately dissolved in nutrient solution 1 in varying amounts, and single fronds in the above solutions were exposed to ultraviolet for not longer than three minutes. The tabulated results are given below.

Note.--With all subsequent work on sterilization of Lemna, the technic already given is changed in only one detail, namely, the plant is inverted with top in germicidal solution. In this way the solution comes in contact with both top and roots, as does the light from the lamp. The plant is washed thoroughly with sterile distilled water before and after exposure to the light in the germicidal solution.

Germicides	Exposure to ultraviolet light	Growth of plant in sterile solution 1 &	Sterility
1% C_2H_5OH in Sol. 1	3 min. 1 frond	Grew	Non-sterile
5% " " " "	3 " "	"	" "
10% " " " "	3 " "	Died	" "
20% " " " "	3 " "	"	Sterile
30% " " " "	3 " "	"	"
5% CH_3OH " " "	3 " "	Grew	Non-sterile
15% " " " "	3 " "	"	" "
30% " " " "	3 " "	Died	Sterile
50% " " " "	3 " "	"	"
5% C_3H_7OH " " "	3 " "	"	Non-sterile
15% " " " "	3 " "	"	Sterile
30% " " " "	1 " "	"	"
1-50 HCHO " " "	3 " "	"	"
1-100 " " " "	3 " "	"	"
1-200 " " " "	3 " "	Grew	Non-sterile
1-500 " " " "	3 " "	"	" "
1-10 $CuSO_4$ " " "	3 " "	Died	" "
1-20 Phenol " " "	3 " "	"	Sterile
1-50 " " " "	3 " "	"	"
1-100 " " " "	3 " "	"	Non-sterile
1-200 " " " "	3 " "	"	" "
1-500 " " " "	3 " "	Grew	" "
1-1000 " " " "	3 " "	"	" "
1-100 $KMnO_4$ " " "	3 " "	Died	" "
1-40000 $HgCl_2$ " " "	3 " "	"	Sterile
1-60000 " " " "	3 " "	"	"
1-100000 " " " "	3 " "	"	"
1-150000 " " " "	3 " "	Grew	Non-sterile
1-200000 " " " "	3 " "	"	" "
1-10000 $AgNO_3$ " " "	3 " "	Died	Sterile
1-20000 " " " "	3 " "	"	"
1-40000 " " " "	3 " "	"	Non-sterile
1-60000 " " " "	3 " "	"	" "
1-1000 $AuCl_3$ in " "	3 " "	"	Sterile
1-2000 " " " "	3 " "	"	"
1-5000 " " " "	3 " "	"	Non-sterile
1-10000 " " " "	3 " "	Grew	" "
1-20000 " " " "	3 " "	"	" "
1-2000 $Ba(OH)_2$ " "	3 " "	Died	Sterile
1-5000 " in "	3 " "	"	"
1-10000 " " "	3 " "	"	Non-sterile
1-20000 " " "	3 " "	Grew	" "
1-40000 " " "	3 " "	"	" "
1-100 $Pb(NO_3)_2$ " "	3 " "	"	" "
1-100 $Th(NO_3)_2$ " "	3 " "	"	" "
1-100 $U(NO_3)_2$ " "	3 " "	"	" "
1-100 Mercuro-chrome " "	3 " "	Died	" "

Germicides	Exposure to ultraviolet light	Growth of plant in sterile solution 1 Δ	Sterility
1-200 Acri- in Sol. 1 flavine	3 min. 1 frond	Died	Non-sterile
1-80 HCl " "	3 " "	"	" "
1-100 Chloro-" " amine-T	3 " "	"	" "
Chlorosacch- arin Sat. " "	3 " "	Grew	" "
1-80 KOH " "	3 " "	Died	Sterile
1-200 " " "	3 " "	"	"
1-500 " " "	3 " "	Grew	Non-sterile
1-1000" " "	3 " "	"	" "
1-2000" " "	3 " "	"	" "

Δ Solution adjusted to pH of 4.8, and 1:10000000 manganese added.

Germicide	Exposure to ultraviolet	Growth of plant in Sol. 1 Δδ	Sterility
1-2 H_2O_2 [x]	in Sol. 1:3 min.	Died	Sterile
1-5 H_2O_2	" " :3 " 1 frond:	"	"
1-10 "	" " :3 " " :	"	Non-sterile
1-20 "	" " :3 " " :	"	" "
1-40 "	" " :3 " " :	"	" "
1-80 "	" " :3 " " :	Grew	" "
1-160 "	" " :3 " " :	"	" "
1-500 ICl_3	" " :3 " " :	Died	Sterile
1-1000 "	" " :3 " " :	"	"
1-5000 "	" " :3 " " :	Grew	Non-sterile
1-10000"	" " :3 " " :	"	" "
Furfural, Sat.	" " : -- -- :	--	--
" 1-1	" " :3 " " :	Died	Sterile
" 1-2	" " :3 " " :	"	"
" 1-4	" " :3 " " :	"	"
" 1-8	" " :3 " " :	"	Non-sterile
" 1-16	" " :3 " " :	Grew	" "
" 1-32	" " :3 " " :	"	" "
1-5000 P[x]Tolyl	" " :3 " " :	Died	Sterile
$HgNO_3$	: :		
1-10000 "	" " :3 " " :	"	"
1-15000 "	" " :3 " " :	"	"
1-20000 "	" " :3 " " :	Grew	Non-sterile
1-30000 "	" " :3 " " :	"	" "
1-50000[x] "	" " :3 " " :	"	" "
1-5000 phenyl	" " :3 " " :	Died	Sterile
$HgNO_3$	: :		
1-10000 "	" " :3 " " :	"	"
1-15000 "	" " :3 " " :	"	"
1-20000 "	" " :3 " " :	Grew	Non-sterile
1-30000 "	" " :3 " " :	"	" "
1-60000[x] "	" " :3 " " :	"	" "
1-20000[x] Ethyl	" " :3 " " :	Died	Sterile
$HgNO_3$	: :		
1-30000 "	" " :3 " " :	"	"
1-60000 "	" " :3 " " :	"	Non-sterile
1-100000[x] "	" " :3 " " :	Grew	" "
1-15000 Methyl	" " : 3 " " :	Died	Sterile
$HgNO_3$	: :		
1-20000 "	" " :3 " " :	"	"
1-30000 "	" " :3 " " :	"	"
1-60000 "	" " :3 " " :	Grew	Non-sterile
1-100000 "	" " :3 " " :	"	" "
1-100 K_2HgI_4	" " :3 " " :	Died	Sterile
1-1000 "	" " :3 " " :	"	"
1-5000 "	" " :3 " " :	"	"

Germicide	Exposure to ultraviolet	Growth of plant in Sol. 1 Δδ	Sterility
1-10000 K_2HgI_4 in Sol.1	3 min. 1 frond	Grew	Sterile
1-20000 " " "	3 " "	"	Non-sterile
1-40000 " " "	3 " "	"	" "
$CaOCl_2$ Saturated " "	5 sec. "	"	" "
	20 " "	"	" "
	30 " "	"	" "
	45 " "	"	Sterile
	60 " "	"	"
	90 " "	"	"
	120" "	Died	"
	150" "	"	"
	180" "	"	"

δContaining 1 gram of solid bacto-nutrient agar per liter of solution.

ΔBacto-nutrient agar is used here instead of Solution 1 in order to avoid sterilizing two fronds. Growth and sterility are ascertained with one plant. pH not adjusted.

mCompounds secured through courtesy of I.B. Johns, Department of Plant Chemistry.

xH_2O_2 is 30%.

From the tabulated results, it may be seen that none of the germicidal agents used sterilized Lemna, without killing it, excepting in the case of the compounds, K_2HgI_4 and $CaOCl_2$. While several of the compounds were sufficiently germicidal to kill all micro-organisms in dilute solutions, they also killed the plant. Some of these substances which were the most effective germ killers and the least harmful to Lemna, and especially the last two listed, were further investigated. The concentrations of these materials were used which, as shown in the above list, were least harmful to the plant and the most efficient as germicides. In these solutions single fronds were exposed to ultraviolet light as before. Below are the tabulated results.

Germicide	Exposure to Ultraviolet light and germicide	Growth of plant in sterile solution 1 Δ	Sterility
20% C_2H_5OH in solution 1	5 fronds for 3 min.	Those that grew	Non-sterile
20% " " "	5 w.buds " "	" " "	" "
1-100 HCHO " "	5 fronds " "	" " "	" "
1-100 " " "	5 w.buds " "	" " "	" "
1-50000 $HgCl_2$ "	5 fronds " "	" " "	" "
1-50000 " " "	5 w.buds " "	" " "	" "
1-40000 $AgNO_3$ "	5 fronds " "	" " "	" "
1-40000 " for "	5 w. buds " "	" " "	" "
1-10000 $AuCl_3$ " "	5 fronds " "	" " "	" "
1-10000 " " "	5 w.buds " "	" " "	" "
1-10000 K_2HgI_4 " "	5 fronds " "	(2 grew out	(2 sterile
1-10000 " "	5 w.buds " "	(1 w.buds grew/	(1 "
1-300 KOH in solution 1	5 fronds " "	Those that grew	Non-sterile
1-300 " " "	5 w.buds " "	" " "	" "
1-3000 ICl_3 " "	5 fronds " "	" " "	" "
1-3000 " " "	5 w.buds " "	" " "	" "
1-4 Sat.furfural in sol. 1	5 fronds " "	" " "	" "
1-4 " " " "	5 w.buds " "	" " "	" "
1-15000 p-tolyl $HgNO_3$ in sol.1	5 fronds " "	" " "	" "
1-15000 " " " "	5 w.buds " "	" " "	" "
1-15000 phenyl $HgNO_3$ " "	5 fronds " "	" " "	" "
1-15000 " " " "	5 w.buds " "	" " "	" "
1-60000 ethyl $HgNO_3$ " "	5 fronds " "	" " "	" "
1-60000 " " " "	5 w.buds " "	" " "	" "
1-60000 methyl $HgNO_3$ " "	5 fronds " "	" " "	" "
1-60000 " " " "	5 w.buds " "	" " "	" "

Note. Fronds are used instead of winter buds for sterilization since they are more easily handled.

ΔContaining 1 gr. solid bacto-nut. agar per liter of solution.

Germicide	Exposure to germicide	Growth of plant in sterile solution 1 Δ	Sterility
20% C_2H_5OH in sol. 1	1 frond for 15 sec.	Died	Non-sterile
20% " " "	1 " " 30 "	"	" "
20% " " "	1 " " 60 "	"	" "
20% " " "	1 " " 120 "	"	" "
20% " " "	1 " " 180 "	"	" "
5% HCHO " "	1 " " 15 "	Grew	" "
5% " " "	1 " " 30 "	"	" "
5% " " "	1 " " 60 "	"	Sterile
5% " " "	1 " " 120 "	Died	"
5% " " "	1 " " 180 "	"	"
1-5000 $HgCl_2$ in"	1 " " 15 "	Grew	Non-sterile
1-5000 " "	1 " " 30 "	"	" "
1-5000 " "	1 " " 60 "	"	" "
1-5000 " "	1 " " 120 "	"	" "
1-5000 " "	1 " " 180 "	Died	Sterile
1-5000 $AgNO_3$ "	1 " " 15 "	Grew	Non-sterile
1-5000 " "	1 " " 30 "	"	" "
1-5000 " "	1 " " 60 "	"	Sterile
1-5000 " "	1 " " 120 "	"	Non-sterile
1-5000 " "	1 " " 180 "	Died	Sterile
1-1000 K_2HgI_4 "	1 " " 15 "	Grew	"
1-1000 " "	1 " " 30 "	"	"
1-1000 " "	1 " " 60 "	"	"
1-1000 " "	1 " " 120 "	Died	"
1-1000 " "	1 " " 180 "	"	"
1-1000 $AuCl_3$ "	1 " " 15 "	Grew	Non-sterile
1-1000 " "	1 " " 30 "	"	" "
1-1000 " "	1 " " 60 "	"	Sterile
1-1000 " "	1 " " 120 "	"	"
1-1000 " "	1 " " 180 "	Died	"

Δcontaining 1 gram solid bacto-nutrient agar per liter of solution.

Germicide	Exposure to germicide	Growth of plant in sterile solution 1 Δ	Sterility
Sat. furfural in sol. 1	1 frond for 15 sec.	Died	Non-sterile
" " " "	1 " " 30 "	"	" "
" " " "	1 " " 60 "	"	" "
" " " "	1 " " 120 "	"	" "
" " " "	1 " " 180 "	"	" "
1-5000 p-tolyl $HgNO_3$ in sol. 1	1 " " 15 "	"	Sterile
1-5000 " " " sol. 1	1 " " 30 "	"	"
1-5000 " " " sol. 1	1 " " 60 "	"	"
1-5000 " " " sol. 1	1 " " 120 "	"	"
1-5000 " " " sol. 1	1 " " 180 "	"	"
1-5000 phenyl $HgNO_3$ in sol. 1	1 " " 15 "	"	"
1-5000 " " " sol. 1	1 " " 30 "	"	"
1-5000 " " " sol. 1	1 " " 60 "	"	"
1-5000 " " " sol. 1	1 " " 120 "	"	"
1-5000 " " " sol. 1	1 " " 180 "	"	"
1-5000 ethyl $HgNO_3$ " sol. 1	1 " " 15 "	"	"
1-5000 " " " sol. 1	1 " " 30 "	"	"
1-5000 " " " sol. 1	1 " " 60 "	"	"
1-5000 " " " sol. 1	1 " " 120 "	"	"
1-5000 " " " sol. 1	1 " " 180 "	"	"

Germicide	Exposure to germicide	Growth of plant in sterile solution 1 Δ	Sterility
1-5000 methyl $HgNO_3$ in sol. 1	1 frond for 15 sec.	Died	Sterile
1-5000 " " " "	1 " " 30 "	"	"
1-5000 " " " "	1 " " 60 "	"	"
1-5000 " " " "	1 " " 120 "	"	"
1-5000 " " " "	1 " " 180 "	"	"
Sat.hexyl resorcinol " "	1 " " 15 "	"	Non-sterile
" " " " "	1 " " 30 "	"	" "
" " " " "	1 " " 60 "	"	" "
" " " " "	1 " " 120 "	"	" "
" " " " "	1 " " 180 "	"	" "
5% HCHO in sol. 1	10 " " 60 "	Those that grew were	" "
1-5000 $HgCl_2$ in sol. 1	10 " " 60 "	" " " "	" "
1-5000 $AgNO_3$ " "	10 " " 60 "	" " " "	" "
1-1000 K_2HgI_4 " "	10 " " 60 "	" " " "	2 Sterile
1-1000 $AuCl_3$ " "	10 " " 60 "	" " " "	Non-sterile
1-10000 p-tolyl $HgNO_3$ in sol.1	10 " " 60 "	" " " "	" "
1-10000 phenyl $HgNO_3$ in sol.1	10 " " 60 "	" " " "	" "
1-10000 ethyl " " "	10 " " 60 "	" " " "	" "
1-10000 methyl " " "	10 " " 60 "	" " " "	" "
Sat. $CaOCl_2$ in sol. 1	100 " "10-60 "	3 grew and were	Sterile

Δ Containing 1 gram solid bacto-nutrient agar per liter of solution.

All fronds and winter buds which have thus far been obtained sterile and have grown, finally turned yellow in the above liquid bacto-nutrient medium and died. They developed chlorophyll at first, but subsequent generations developed without green color. There seemed, then, a relation between chlorophyll formation in green plants and micro-organisms. This was soon shown to be incorrect, for non-sterile Lemna in the above medium, produced yellow daughter buds and soon died as did the sterile plants. The pH of the above solution which is 6.6, was adjusted to 4.8, and non-sterile plants added to it. After a few days growth, all buds that formed were green. It was later shown that sterile Lemna would grow, reproduce and continue green in sterile solution 1 containing 1 gram of solid bacto-nutrient agar per liter of solution, providing the pH was adjusted to 4.8.

Some of the fronds which had not produced bacterial growth in the above medium, but which had died because of the high pH, were macerated and transferred to a solid bacto-nutrient medium, and after several days, many pin point colonies were observed. These particular plants were sterile then on the outside and non-sterile on the interior.

The solution used to grow these fronds after treating them with a germicidal agent was prepared as follows:

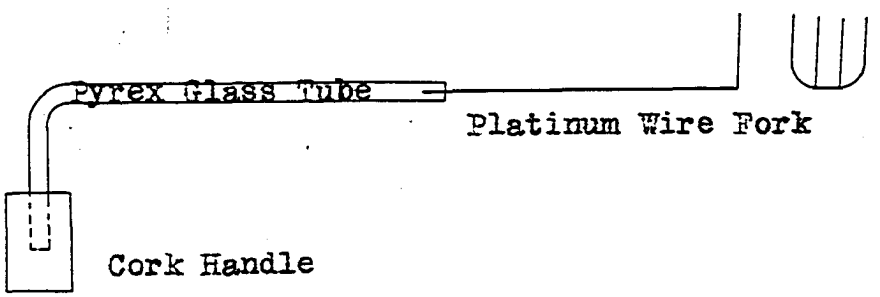
1 liter solution 1 containing 1:10000000 parts of manganese.

1 gram Bacto-nutrient agar (dehydrated)

The above mixture is brought to a boil, pH adjusted to 4.8, and is sterilized at 20 pounds pressure for 15 minutes. For solid agar plates and tubes, 23 grams of the bacto-nutrient agar are dissolved in one liter of distilled water, brought to a boil, and sterilized as usual.

STERILIZATION OF LEMNA

A non-sterile Lemna frond which had been growing in modified solution 1 was washed well with sterile distilled water, contained in a unit similar to those shown in Figure 1. The water in a two liter Erlenmeyer flask, and the rest of the unit were sterilized separately, carefully assembled afterwards, stoppers paraffined, and cotton plugs inserted in the burette and flask vents in order to avoid contamination. The frond was washed by allowing 20 drops of this water to fall on to it from the burette of the unit. The plant was then transferred by means of a sterile platinum wire to a small Erlenmeyer flask containing a saturated solution of $CaOCl_2$, which had been freshly prepared. The instrument used in holding the Lemna while washing it and in transferring it, is illustrated below:



The small Erlenmeyer flask contained just enough saturated solution of $CaOCl_2$ to keep the bottom of the plant from touching the flask. By careful manipulation the frond was placed in this solution in an inverted position after having washed it with sterile water. By this operation, the entire surface of the plant came in contact with the solution. After about 30 seconds it was removed from the flask by means of the above sterile instrument, washed again with about 20 drops of sterile water, and transferred immediately to a cotton stoppered flask containing sterile modified solution 1 with 1 gram of bacto-nutrient agar. The technic usually employed in the bacteriological laboratory was observed in making these transfers, 400 plants were treated according to the above procedure, except that K_2HgI_4 was used for about 200 instead of a saturated solution of $CaOCl_2$. These two compounds were the only germicidal agents previously used successfully, and while the former compound proved to be the better germicide, it was the more toxic to the plants.

After the plants were treated as described above, they were incubated at 25°C. for five or six days away from direct sunlight, then later in the sunlight, until it was certain which plants were living and sterile. These were then carefully transferred to fresh medium. Green buds soon developed from the mother plant which had been almost completely killed by the germicide. From the 400 single plants treated there were only 30 which lived and were sterile, the others were

non-sterile and dead. Those that were sterile and grew, died soon after developing green buds. The original frond was killed by the germicide but the green bud which during treatment was in the interior of the plant or nearly so, later grew out. The bud before emerging is probably sterile, so when all the frond is sterilized except the bud, the latter is not seriously harmed by the treatment. Thus, it later emerges and is sterile.

The plants believed sterile were kept in several different flasks, so that if one container became contaminated, others might be spared. Transfers to fresh sterile liquid bacto-nutrient medium in small containers were made twice a week, until there were several hundred plants at which time 8-2 liter Erlenmeyer flasks were substituted.

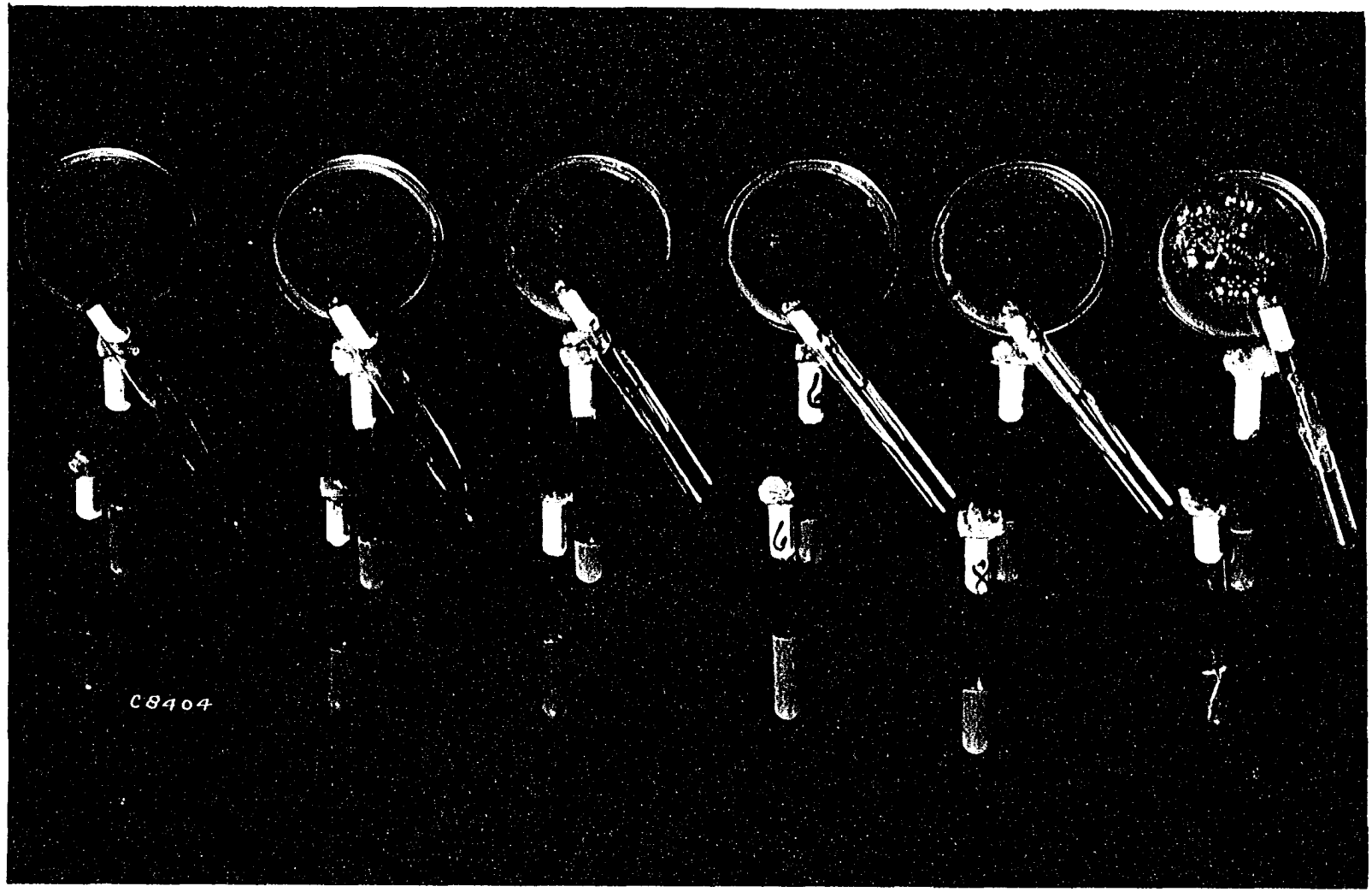
For the transfer work at this point and during the rest of this work a small room 8'x5'x8' made of composition board was employed. It contained a door, a window, a gas burner, an electric light, shelves and two small holes in the top which were filled with cotton. The walls, ceiling and floor of this room before use were washed with 1:500 $HgCl_2$ solution and its interior was sprayed with the same solution which was allowed to settle. Before spraying, all of the solutions to be changed and those instruments which do not come in contact with the nutrient solution during the transfer were placed inside. The sterile transfers were then made by the writer,

who had previously undressed and dampened his hand, face and hair. Since the temperature of the room was held between 30°C. - 38°C. there was sufficient body moisture to prevent dust. The sterility of this room was effected after spraying with $HgCl_2$ solution, and it was found that out of several tests not more than five micro-organisms fell on an agar plate, which had been exposed for one-half hour to the room interior. Wearing a wet laboratory coat, the writer attempted to make these sterile transfers in the above room, but serious contamination resulted as was shown by the subsequent bacterial growth on a nutrient agar plate which had been exposed to the room's atmosphere, consequently, the technic already described was used instead.

When transferring the plants for the first time, from these 8-2 liter Erlenmeyer flasks to fresh sterile liquid, bacto-nutrient medium, some Lemna were taken from each flask and placed on a solid nutrient agar plate. After incubation, only one plate was perfectly sterile. This sterility test was checked and the results showed, as before, that the plants in only one flask were sterile. These were then divided into eight equal parts and transferred to fresh sterile medium. At the next transfer, a test revealed that the plants in the eight flasks were sterile. Maceration showed no bacterial infection.

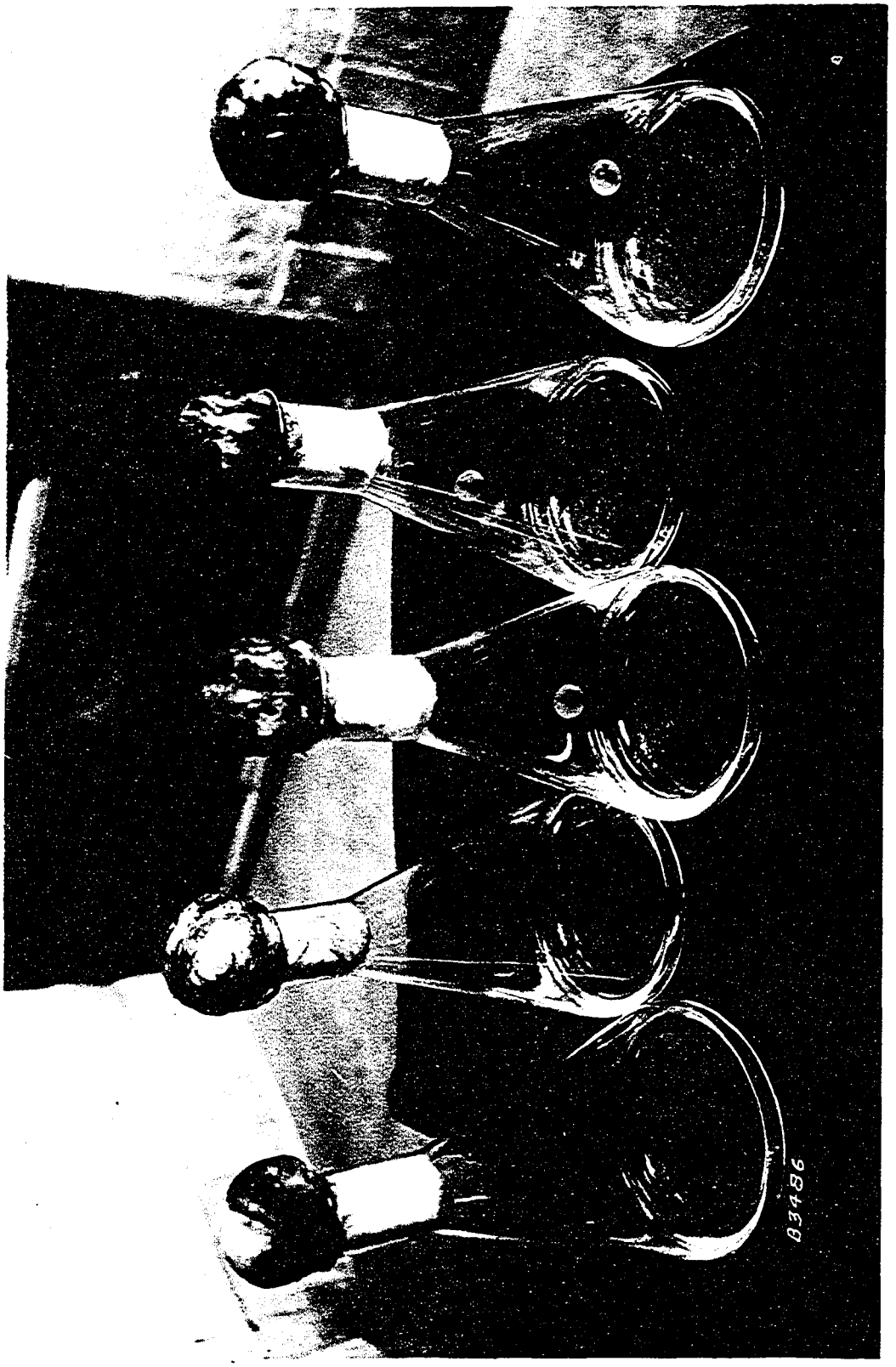
The sterility of these plants was further demonstrated by inoculating one from each flask into the following bacto-

nutrient media: bacto-nutrient agar, dextrose agar, bacto-nutrient broth, bacto-nutrient agar + 5 grams NaCl per liter. After several days, no bacterial growth was noticed in any of the above media. Figure 4 shows these sterile tests compared to the non-sterile plant. Figure 5 shows the sterile green plants in Erlenmeyer flasks, and Figure 6 shows the non-sterile plants in large crystallizing dishes.



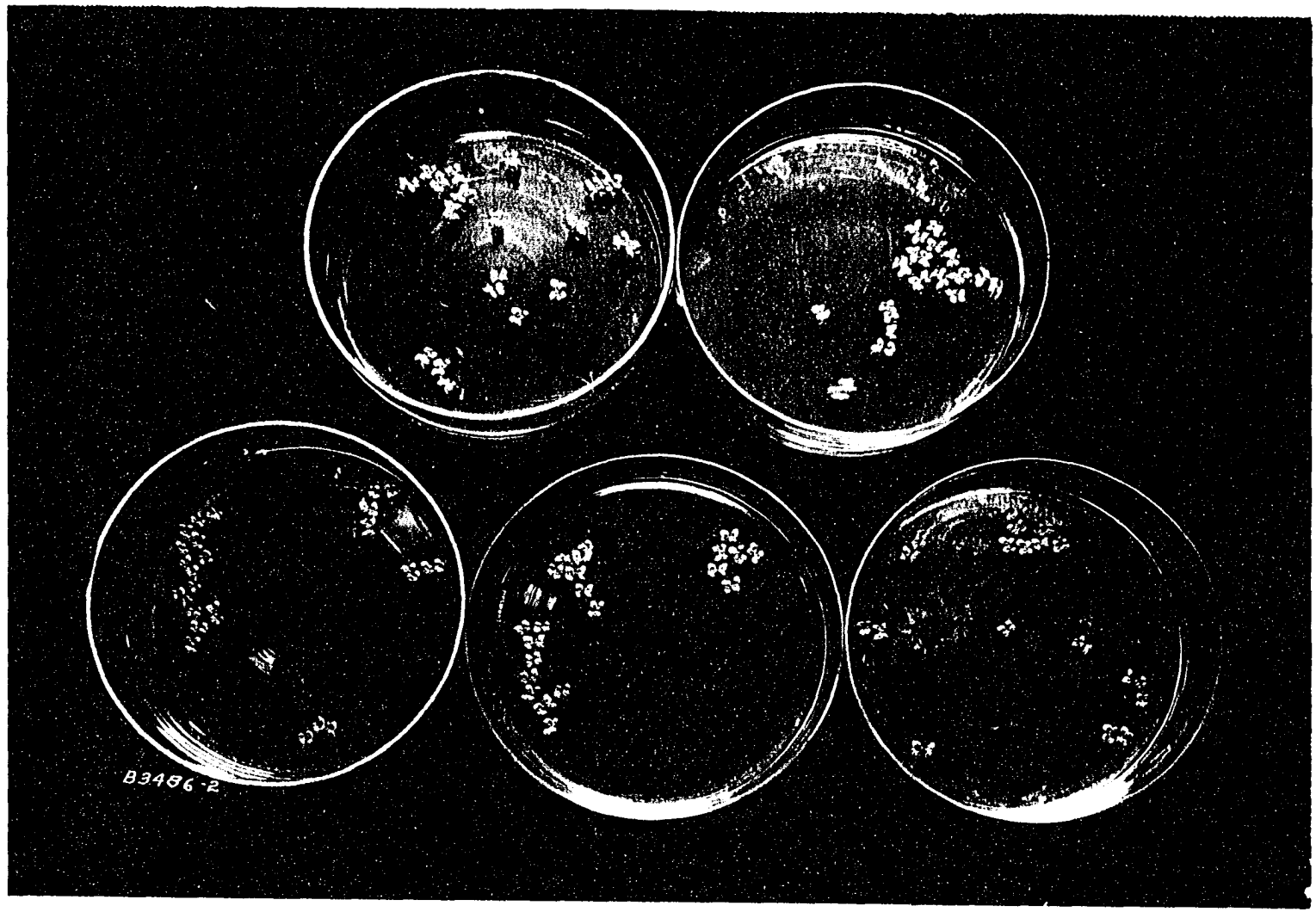
Tests in Different Media To Show Sterility of Lemna

Figure 4



Steri'le Lemna Growing in Modified Solution I

Figure 5



Non-Sterile Lemna Growing in Modified Solution I

Figure 6

Attempts were made to make Lemna which had been treated with various germicides grow in sterile inorganic solution 1 as is shown in the first part of the tabulated results, but without success. This was again attempted by treating 100 single fronds with a saturated $CaOCl_2$ solution and instead of transferring the plants to the liquid bacto-nutrient medium, they were inoculated into sterile modified solution 1. After five or six days in a dark place, they were exposed to direct sunlight, until it was definitely known which would grow. The growing plants were then transferred to fresh sterile solutions, and after several such transfers they were changed to the liquid bacto-nutrient medium already mentioned in order to test for the presence of bacteria. Some were sterile and grew. This experiment was repeated with similar results, hence it proves that Lemna can be sterilized and grown in sterile solution 1 instead of the bacterial medium. The sterile plants which had been growing in the liquid bacto-nutrient medium were then transferred to sterile modified solution 1. In this inorganic medium sterile Lemna has passed through about 75 generations and continues to look healthy and to reproduce without the addition of organic matter. Tests made from time to time have shown them to be free from micro-organisms.

It has already been proved by Clark and Roller (18), and confirmed by others (3.20,37,47) that the green plant will

grow and reproduce quite satisfactorily without the addition of minute quantities of organic substances, or accessory factors known as auximones. Now, it is evident that micro-organisms are not necessary for the proper nutrition of Lemna plants if their rate of growth and their healthy appearance and their sterility after eight months of continuous growth in a sterile inorganic solution can be used as criteria.

These plants build up their cell constituents of protein, carbohydrates, fats and salts from only the sterile nutrient modified solution 1, containing purified inorganic salts, and triple distilled water and the carbon dioxide from the air. KNO_3 is their chief source of nitrogen from which they synthesize protein. Carbon dioxide from the air, and water from the solution are the chief sources from which the plant can build carbohydrates and fats.

Sterile and non-sterile Lemna are now grown under the same conditions, excepting the modified solution 1 in the first case is sterile, while in the second case it is non-sterile. In the former the plants are designated as sterile stock, while in the latter they are designated as non-sterile stock. The effects of various kinds of organic substances on the plants of these two stockswill be shown later in this thesis. We shall try to determine whether organic matter will stimulate or retard their growth.

THE EFFECTS OF ORGANIC MATTER ON THE GROWTH RATE OF NON-STERILE GREEN PLANTS.

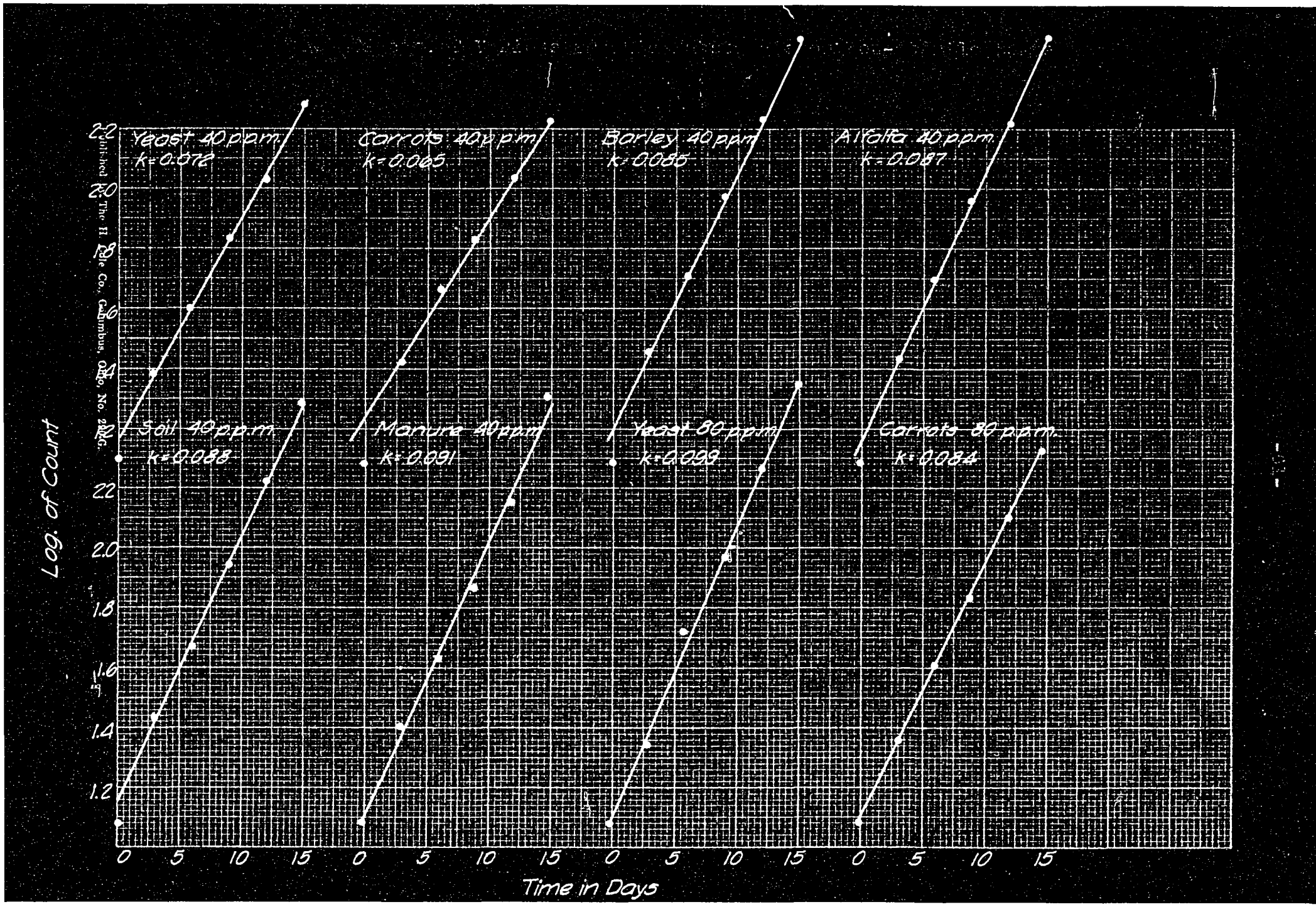
Early in the nineteenth century, humus was regarded as the food of green plants, but the work of de Saussure and Senebier, together with Leibig's address to the British Association in 1840, served to kill the humus theory (36). In France Grandeau, late in the nineteenth century, suggested the possibility that organic substances contribute occasionally to the supply of carbon in the plant, but this was not recognized, however, until modern work showed that organic materials under certain circumstances could be absorbed by the plant and might prove either toxic or beneficial (36). At the present time there is no doubt that the carbon requirement of the green plant can be supplied by the carbon dioxide of the air.

Many investigators have worked on the relationship of organic matter to the growth of the green plant. Among those who have studied this phase of plant nutrition are Schreiner, Shorey, Skinner, and Reed of the Bureau of Soils (38,39,40, 41, 42). These men have isolated some pure organic compounds from the soil, and have found that some were beneficial, some toxic and some ineffective to plants grown in a nutrient solution. Robbins (34) studied the effects of autolyzed yeast and peptone on growth of excised corn root tips in the dark, and found that a 2 per cent solution of glucose in Pfeffer's medium increased the growth of corn root tips when grown under

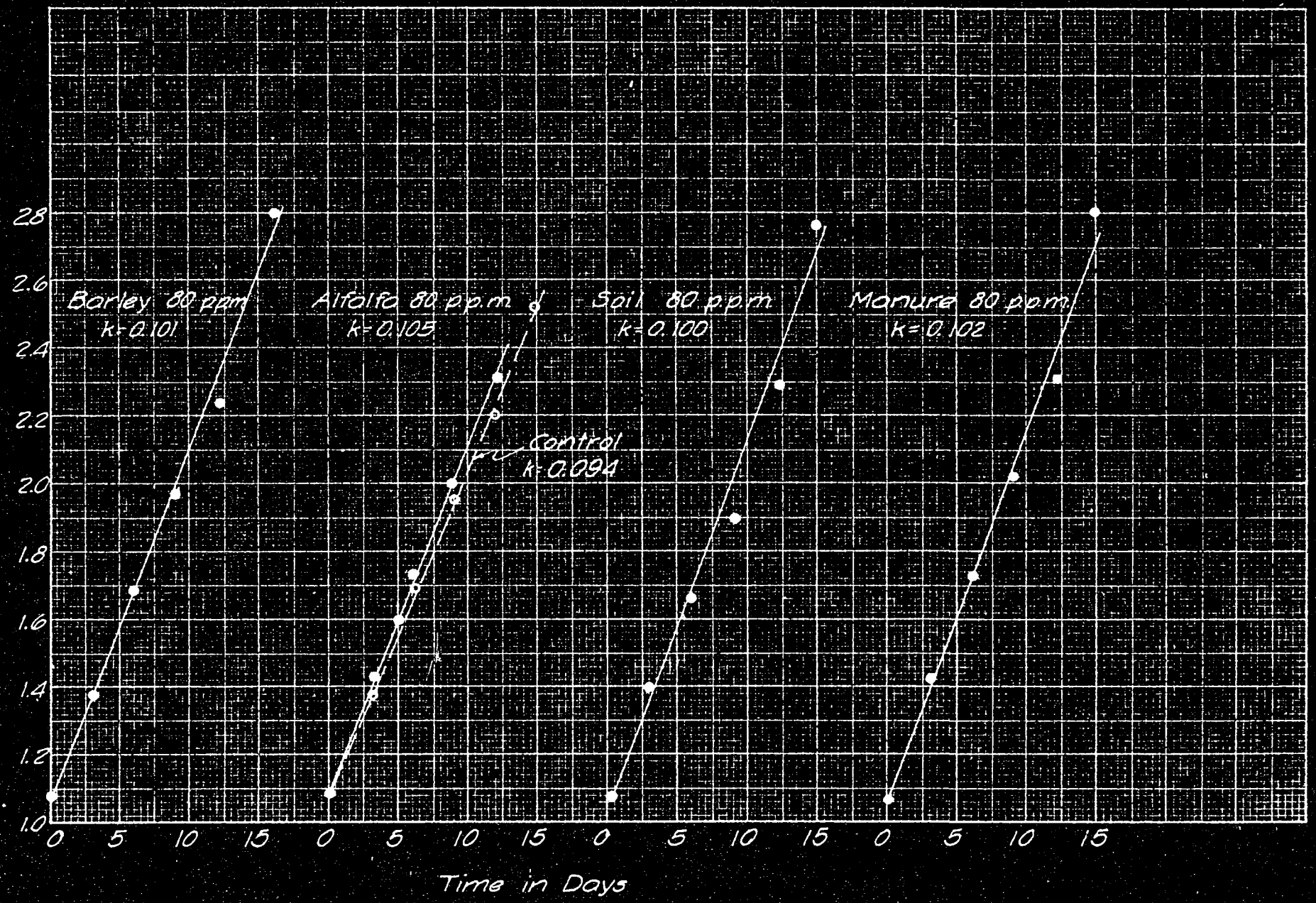
sterile conditions. He also found that peptone and autolyzed yeast acted as stimulants, while creatinine, asparagin and glycocoll were of no nutritive value. Chittenden showed that bacterized peat acted as a stimulant. Breazeale (12) believed that organic substances which come in contact with the soil decompose and compounds result which stimulate plant growth. Rosenheim (35) sterilized rotted peat and inoculated it with a mixed culture of nitrogen-fixing bacteria. This inoculated peat increased the growth of plants grown in pots. Bottomley (4,5,9) found that sterilized cultures of Azotobacter, autolyzed yeast, nucleic acid derivatives, humates, and bacterized peat stimulated the growth of Lemna when grown in Detmer's and Knop's Solutions, in which the plants would not grow without the addition of minute quantities of organic matter. Saeger (37) checked Bottomley's results using autolyzed yeast and an aqueous extract of peat.

After Lemna major had been made to grow by Clark and Roller in a solution containing only crystallized inorganic salts and triple distilled water, we next desired to determine the effects of organic substances upon the growth and reproduction of this plant in solution 1 with pH held constant at 4.8. The materials used were Fleishman's yeast, ripe carrots, ripe barley, cured alfalfa, Carrington loam soil and rotted stable manure. In each case the sample was macerated or ground up, digested with distilled water at 70°C.

for four hours, with occasional shaking, and filtered free of any suspended matter. This extraction was repeated and the filtrates were collected and concentrated in vacuo at 65°C. They were then made up to volume and the concentrations of solid matter was determined for each of the six extracts. The solutions were kept in an ice box to keep down bacterial growth. The technic used in this experiment has been described in the "Methods and Technic" of this thesis. The plan of the experiment with results and growth rate curves are given below.


Yeast 40 p.p.m.
k=0.072
Carrots 40 p.p.m.
k=0.085
Barley 40 p.p.m.
k=0.085
Alfalfa 40 p.p.m.
k=0.087
Soil 40 p.p.m.
k=0.088
Manure 40 p.p.m.
k=0.091
Yeast 80 p.p.m.
k=0.099
Carrots 80 p.p.m.
k=0.084
Log. of Count
Time in Days
2.2
2.0
1.8
1.6
1.4
1.2
0
5
10
15
Published by The H. R. Co., Columbus, O. No. 282

The Effects of Various Organic Substances on Non-sterile Loams



Form B-5

TABLE IV. THE EFFECTS OF VARIOUS ORGANIC SUBSTANCES ON NON-STERILE LEMNA.

Extract	Green weight grams	Moisture weight grams	Dry weight	k	Genl. appearance at end	p.p.m. of extract added	pH of solution 1
x yeast	0.0548	0.0491	0.0052	0.072	a - 2	40	4.8
	0.0566	0.0512	0.0057	0.099	a	80	4.8
x carrots	0.0588	0.0536	0.0053	0.065	a - 3	40	4.8
	0.0599	0.0545	0.0060	0.084	a - 3	80	4.8
x barley	0.0692	0.0642	0.0055	0.085	a - 1	40	4.8
	0.0752	0.0684	0.0069	0.101	a + 1	80	4.8
x alfalfa	0.0657	0.0600	0.0057	0.087	a	40	4.8
	0.0641	0.0582	0.0056	0.105	a + 1	80	4.8
soil	0.0620	0.0577	0.0042	0.080	a	40	4.8
	0.0740	0.0685	0.0056	0.100	a + 1	80	4.8
manure	0.0725	0.0672	0.0049	0.091	a + 1	40	4.8
	0.0878	0.0821	0.0063	0.102	a + 2	80	4.8
control	0.0717	0.0677	0.0054	0.094	a	none	4.8

a = appearance of control.
x = a micro-organic growth was attached to the plant roots which could not be washed free.
pH of extracts adjusted to 4.8
duration of experiment -- 15 days.

In order to obtain the above green weights, fifty plants were taken out of each solution, blotted between filter papers for three minutes, and weighed immediately in weighing bottles. For the dry weights, the weighing bottles and contents were heated in a vacuum oven held at 30 m.m. pressure, and 65°C. for 12 hours. The loss of moisture represents the moisture weights.

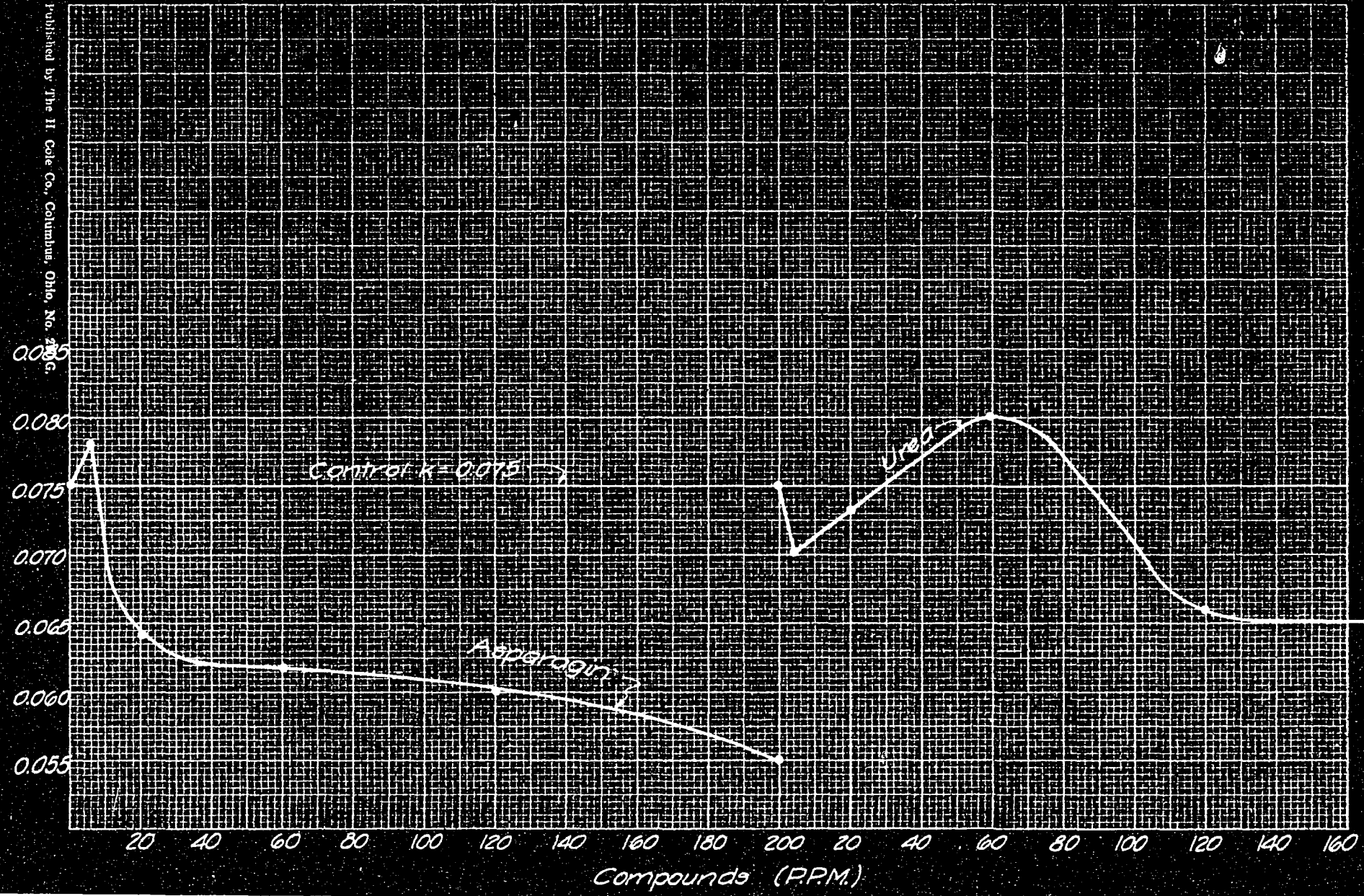
The results in Table IV indicate that the constants (k) for plants grown in solutions containing 40 p.p.m. of the various organic extracts are smaller than that for the control, while the opposite is true for solutions containing 80 p.p.m., excepting in the case of carrots where (k) is smaller for both concentrations than for the control. For this extract, the green weight, moisture weight and (k) showed no increase over those for the control in either concentration, while the dry weight showed stimulation at 80 p.p.m. Increase ingreen weight is noticed with both solutions of manure, and with 80 p.p.m. of soil and barley, while with the other organic substances there is a decrease for both concentrations. For the dry weight, 80 p.p.m. of all extracts and even 40 p.p.m. of alfalfa and barley gave an increase, while the other organic materials gave a decrease. From these results it is noticed that the values for (k) and the dry weights are reasonably well correlated with each other, but not so well with the green weights. Based on the growth rates, the extracts are arranged in the descending order of

their value: alfalfa, manure, barley, soil, yeast and carrots; based on dry weights: barley, manure, carrots, yeast, (alfalfa and (soil ; and based on appearance: manure, soil alfalfa, barley, yeast and carrots.

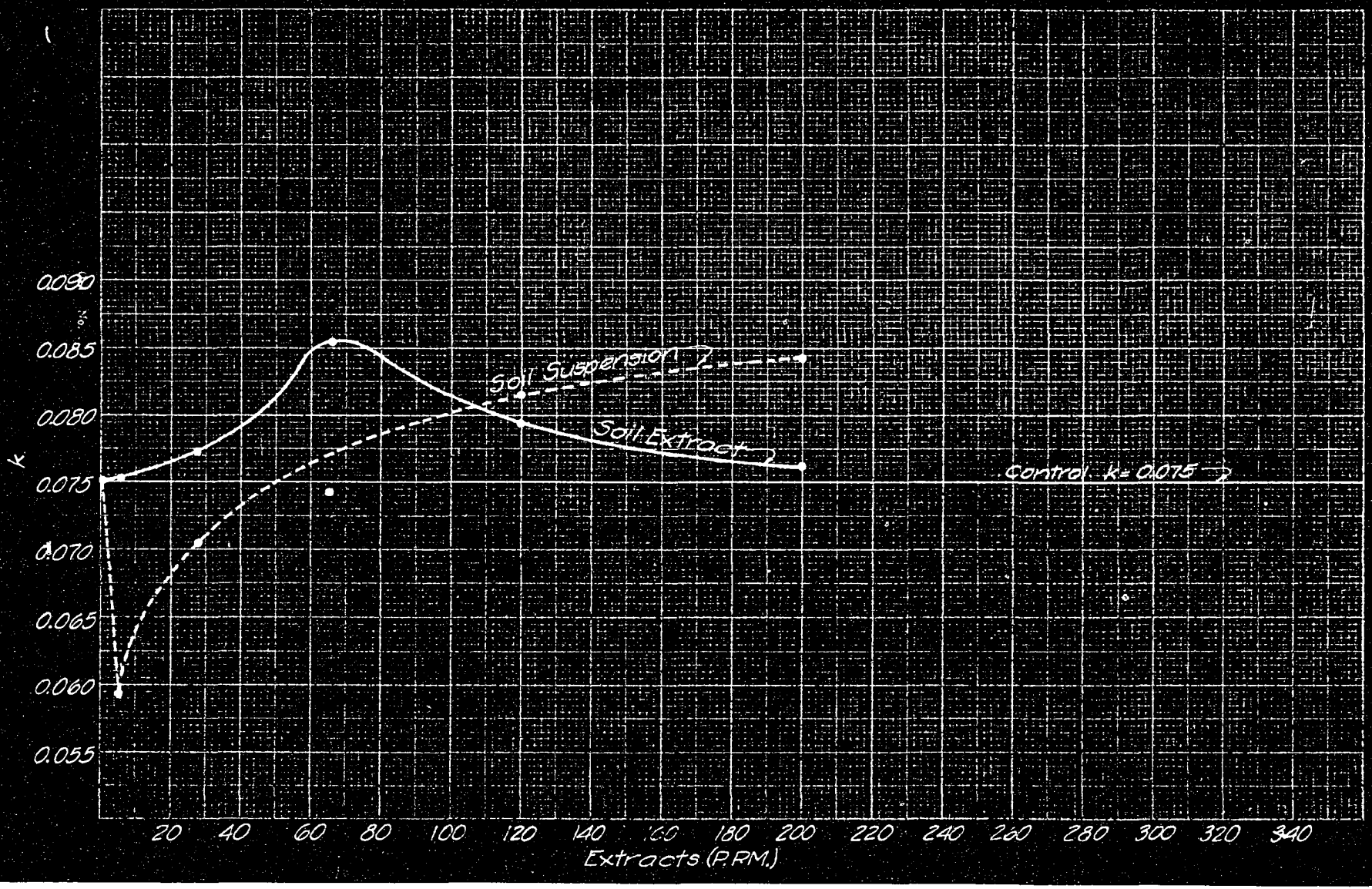
The fact that small concentrations of the above organic extracts depress the growth of plants, while the larger amounts increase growth will perhaps be more fully understood later, when maximum growth rate curves are determined for some of the organic substances just mentioned.

Our next experiment on organic matter and the growth of Lemna was an attempt to obtain an optimum concentration of soil and manure extracts for the green plant, and an attempt to show the effects upon Lemna of some pure organic compounds when added to an inorganic nutrient solution. The methods and technic were the same as those used in the experiment just given, except that Saeger's Solution was used instead of solution 1. The pH was held constant at the optimum, i.e., 4.9 as given in Table II. The following table and graphs show the plan and results of this experiment.

Curves Showing the Optimum Concentrations of Asparagin and Urea for The Growth of Non-sterile Lemna



Curves Showing the Optimum Concentrations of Soil Suspension and Soil Extract for the Growth of Non-sterile Lemna



Curves Showing the Optimum Concentrations of Manure Extract and Manure Suspension for the Growth of Non-sterile Lemna

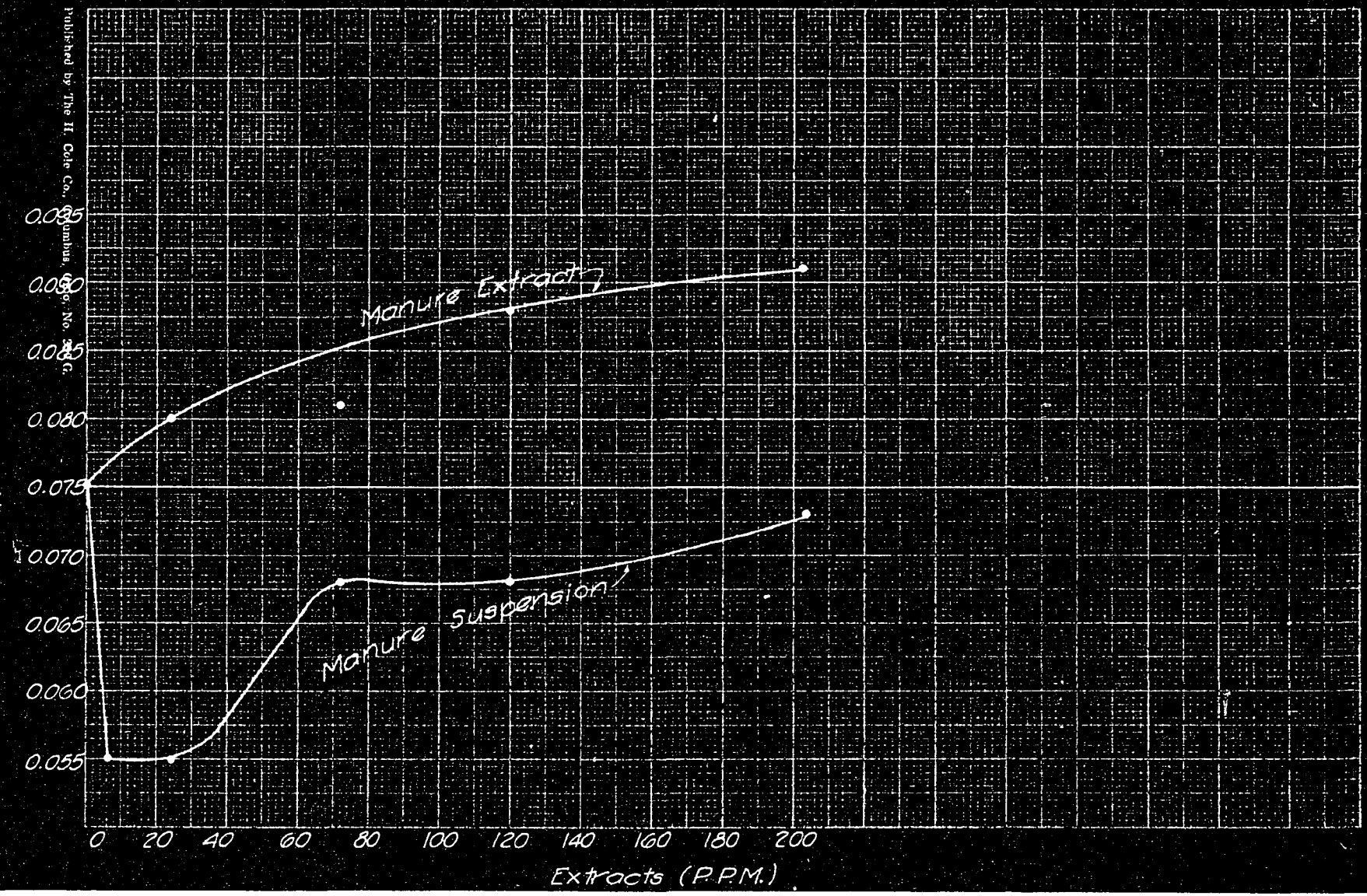


TABLE 5. THE OPTIMUM CONCENTRATIONS OF VARIOUS ORGANIC SUBSTANCES FOR THE GROWTH OF NON-STERILE LEMNA.

No.	p.p.m. Stimulant added	Dry weight	k	Appearance at end	Organic stimulant (The pH of extracts was kept constant at 4.9)
1	4	0.0046	0.078	a	Asparagin x
2	20	0.0049	0.064	a-1	"
3	60	0.0060	0.062	a-4	"
4	120	0.0079	0.060	a-5	"
5	200	0.0106	0.055	Dying	"
1	4	0.0046	0.070	a-1	Urea x
2	20	0.0038	0.073	a-1	"
3	60	0.0044	0.080	a	"
4	120	0.0059	0.066	a-4	"
5	200	0.0055	0.065	a-5	"
1	4	0.0049	0.075	a	Soil extract
2	20	0.0048	0.077	a	" "
3	60	0.0043	0.085	a+1	" "
4	120	0.0056	0.079	a-1	" "
5	200	0.0063	0.076	a-1	" "
1	4	0.0068	0.059	a-4	" susp.in dist. H_2O equiv. to p.p.m. of soil ext.
2	20	0.0063	0.070	a-1	" " " " " " " " " " "
3	60	0.0069	0.074	a-1	" " " " " " " " " " "
4	120	0.0075	0.081	a	" " " " " " " " " " "
5	200	0.0080	0.083	a	" " " " " " " " " " "
1	4	0.0047	0.077	a	Manure extract
2	20	0.0044	0.080	a	" "
3	60	0.0053	0.081	a	" "
4	120	0.0047	0.088	a+1	" "
5	200	0.0049	0.091	a+2	" "
1	4	0.0064	0.055	Dying	" susp. in dist. H_2O equiv. to p.p.m.of manure ext.
2	20	0.0074	0.055	a-4	" " " " " " " " " " "
3	60	0.0056	0.068	a-1	" " " " " " " " " " "
4	120	0.0063	0.068	a-1	" " " " " " " " " " "
5	200	0.0073	0.073	a-1	" " " " " " " " " " "
1	--	0.0055	0.075	a	Control (Saeger's Solution)

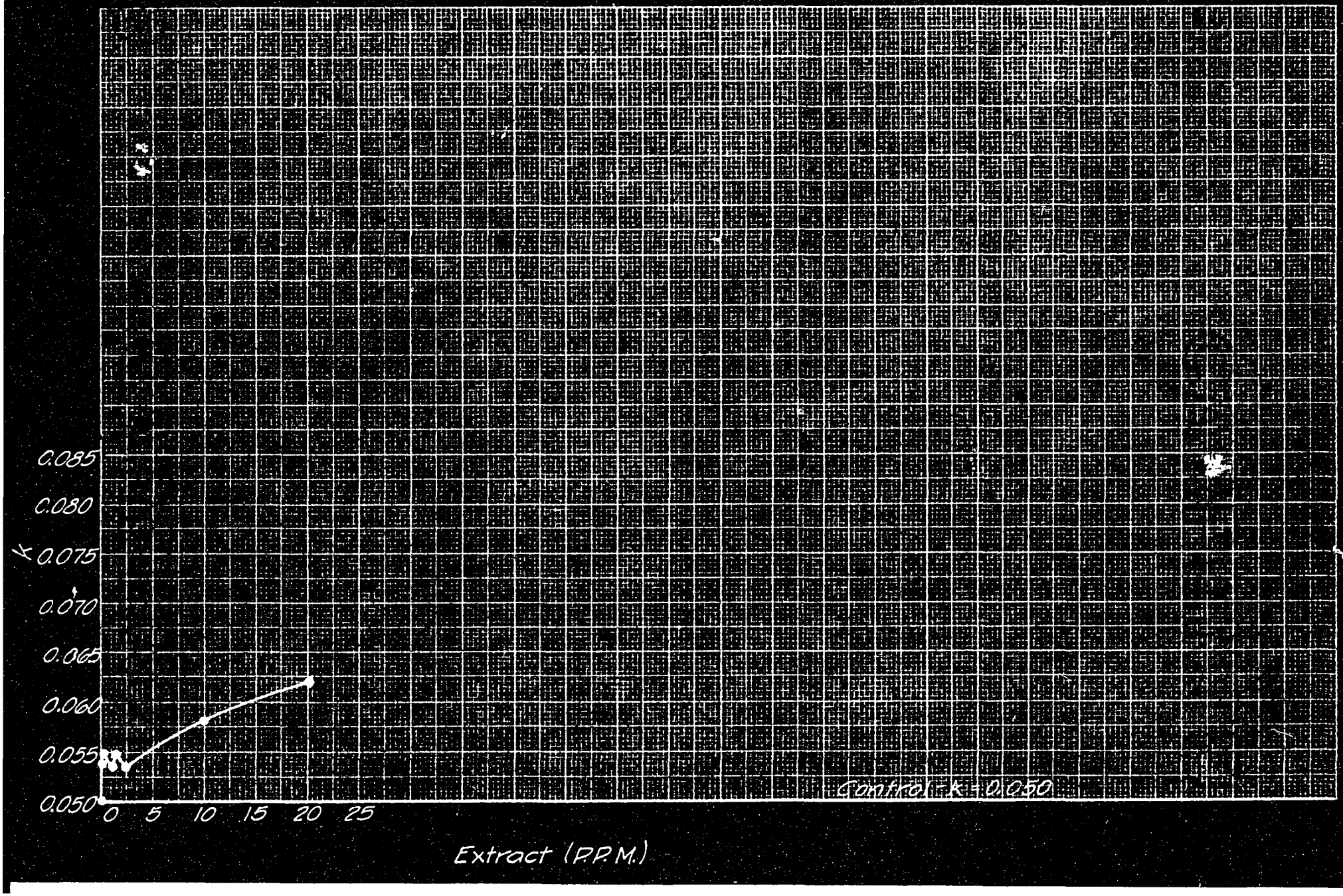
x Micro-organic growth attached to roots which could not be washed free.
Duration of experiment 4 weeks.

The preceding table and curves show that the organic substances used in this experiment stimulated the growth rate of Lemna. Some of the materials, however, were noticeably better than others. Asparagin in the lowest concentration acted as if it were a stimulant, but was decidedly toxic in the higher concentrations. Urea, on the other hand, is toxic when present in large or small amounts, it accelerates the growth of Lemna at 60 p.p.m., which is the optimum as is indicated on the curve. These results were roughly checked by Wolfe (47). The soil extract did not retard the growth in any of the solutions. Its optimum concentration for growth rate was reached at about 70 p.p.m. One notes that manure extract is best of the stimulants used, but its optimum was not reached even at 200 p.p.m. Both the soil and manure suspensions gave results different from those obtained from their extracts. Though the extracts were either stimulants, or ineffective at all concentrations used, the suspensions were either toxic or stimulants, and neither suspension reached an optimum. Perhaps an optimum would have been reached with each material had greater amounts been used. None of the concentrations of the manure suspension was beneficial; with the soil suspension, however, the 120 and 200 p.p.m. stimulated the growth rate of the plant. The growth constants and general appearance of the plants, it is noticed, are closely correlated, while the dry weight values bear no significant relation to the

other results. In any event, as the rates of reproduction change the dry weights do not necessarily change correspondingly.

We wished next to investigate the effects of minute quantities of soil and manure extracts upon the growth of Lemna major. The methods and technic used are the same as in the experiment just described. The following table and graphs show the plan and results of this experiment.

Effects of Minute Quantities of Soil Extract Upon the Growth of Non-sterile Lemna



Effects of Minute Quantities of Manure Extract
Upon the Growth of Non-sterile Lemna

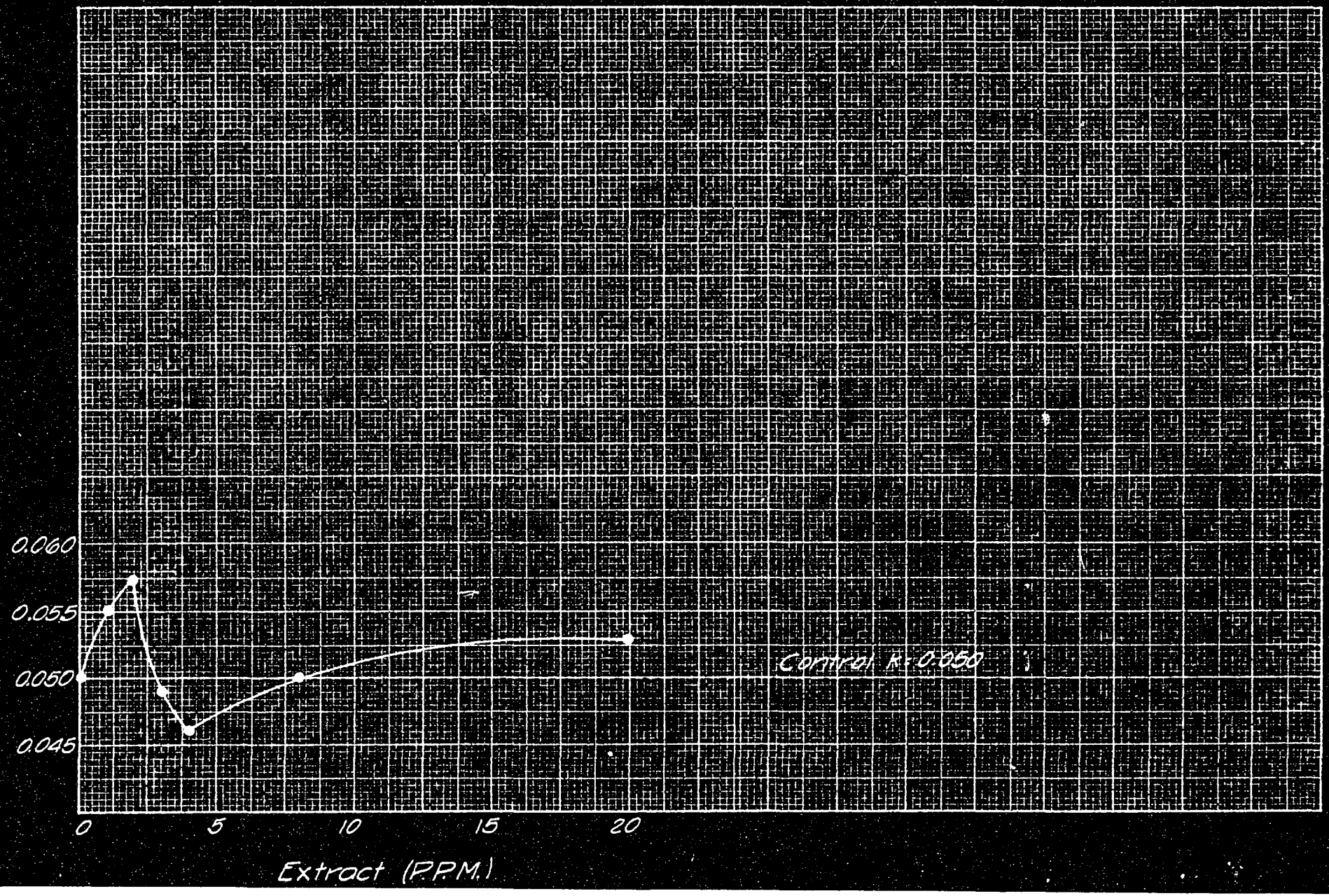


TABLE VI. THE EFFECTS OF MINUTE QUANTITIES OF SOIL AND MANURE EXTRACTS ON THE GROWTH OF NON-STERILE LEMNA.

No.	p.p.m. stimulant added	k	Organic stimulants (The pH of extracts was kept constant at 4.9).
1	0.2	0.054	Soil extract
2	0.5	0.055	" "
3	0.8	0.054	" "
4	1.0	0.055	" "
5	2.0	0.054	" "
6	10.0	0.058	" "
7	20.0	0.062	" "
1	1	0.055	Manure extract
2	2	0.058	" "
3	3	0.049	" "
4	4	0.046	" "
5	8	0.050	" "
6	20	0.053	" "
1	--	0.050	Control (Saeger's Solution)

Duration of experiment 4 weeks.

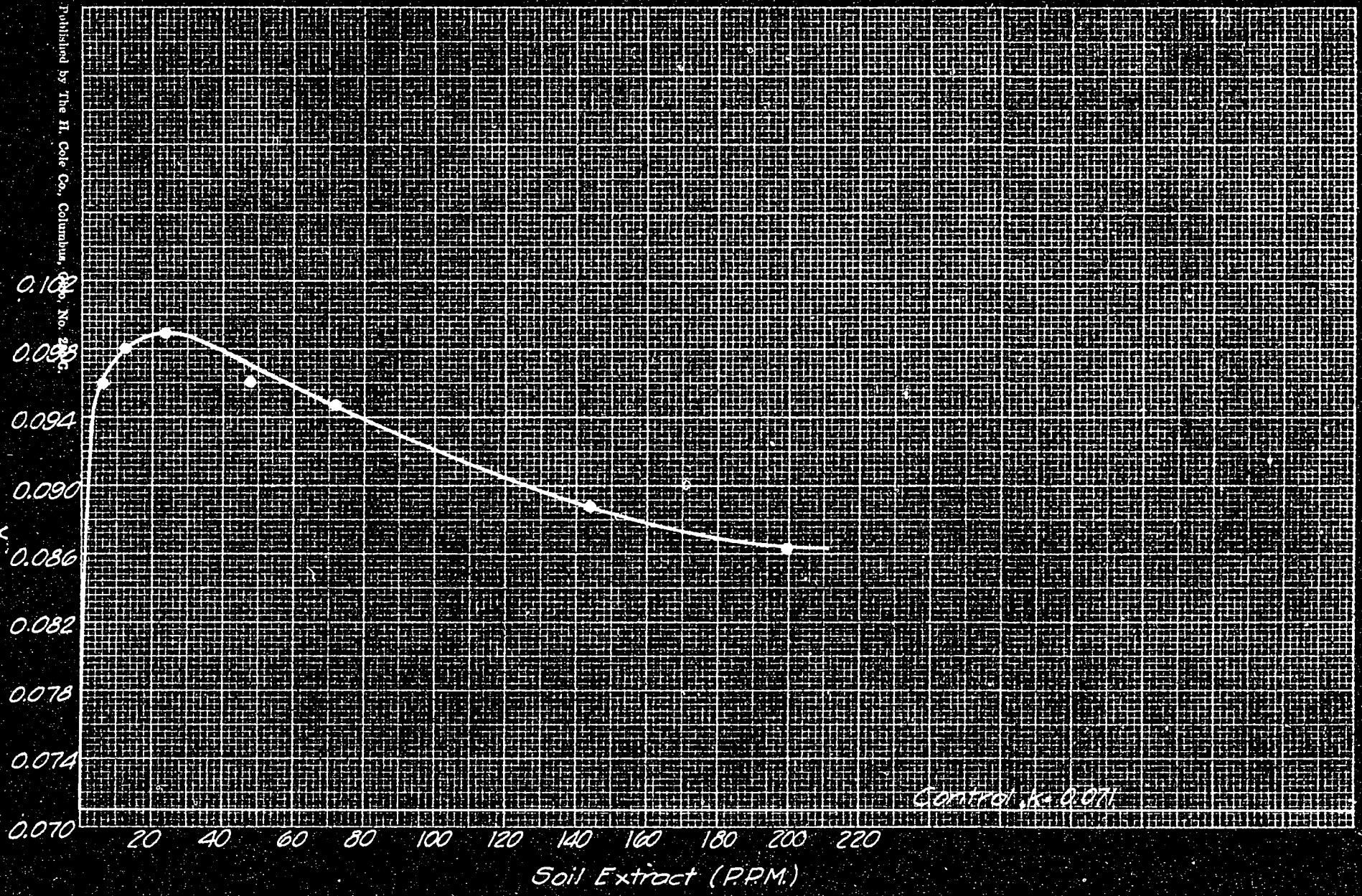
Both soil and manure extracts in minute quantities stimulate the growth rate of the green plant. It is noticed, however, that two concentrations of manure extract give growth constants below that of the control. This produces a curve which apparently has a maximum and a minimum point. The curve indicates also that this extract is a stimulant in very minute and in large quantities, but is toxic somewhere between these concentrations.

Ashby (3), using sterile manure, found that minute quantities increased the growth of Lemna minor, but he did not use larger amounts than 20 p.p.m.

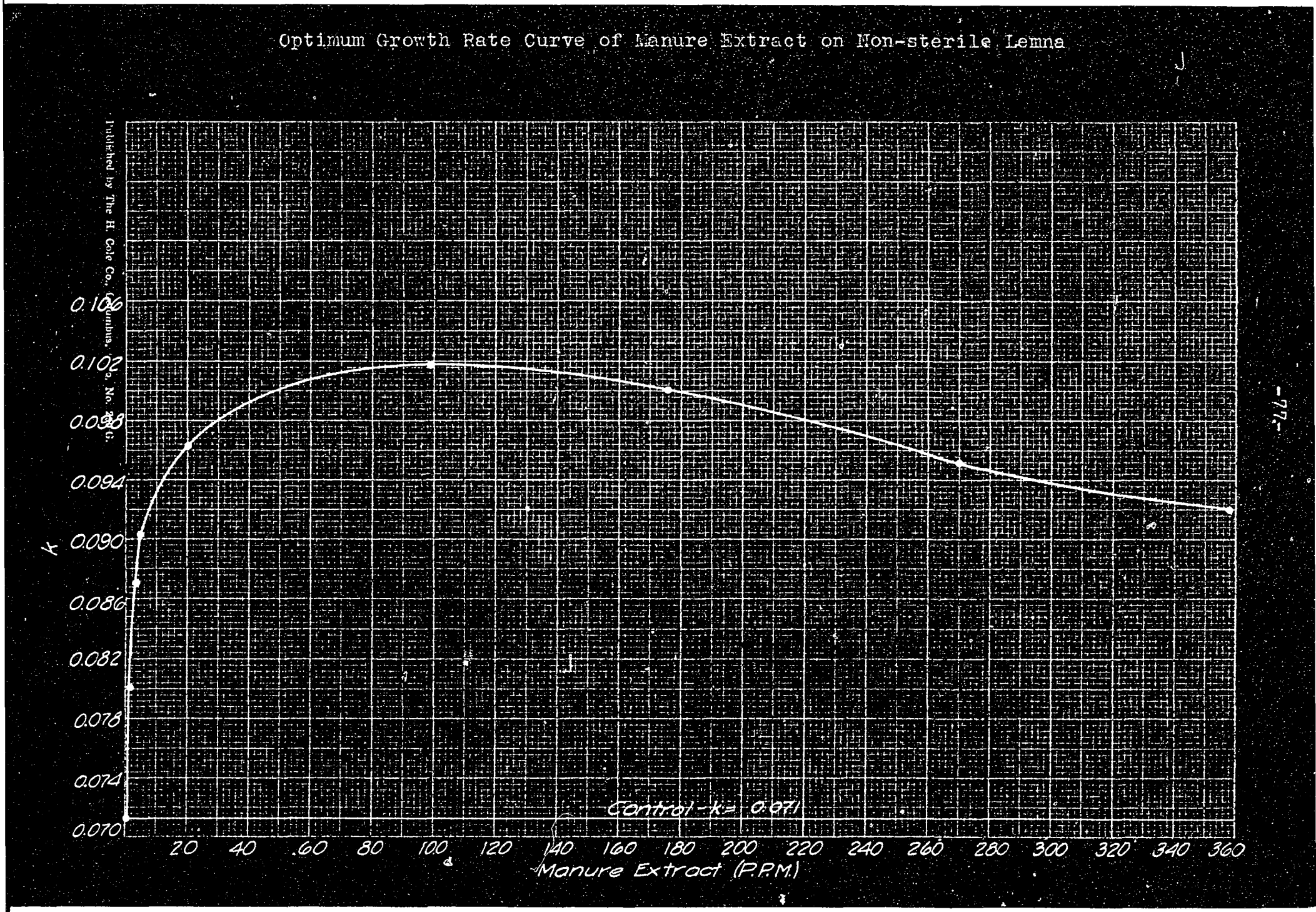
The above graph and table show that the soil extract acts as an accelerator in all concentrations used.

We next attempted to determine the optimum for manure and alfalfa extracts and again to check the optimum for soil extract. The procedure was the same as that in the previous experiment, except that solution 1 was used, and the pH maintained constant at 4.8. The following table and graphs show the plan and the results of this experiment.

Optimum Growth Rate Curve of Soil Extract on Non-Sterile Lemna



Published by The H. Cole Co., Columbus, O. No. 2338


Optimum Growth Rate Curve of Manure Extract on Non-sterile Lemna
Published by The H. Cole Co., Columbus, No. [illegible]
k
0.106
0.102
0.098
0.094
0.090
0.086
0.082
0.078
0.074
0.070
Control - k = 0.071
20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360
Manure Extract (P.P.M.)

Optimum Growth Rate Curve of Alfalfa Extract on Non-sterile Lemna

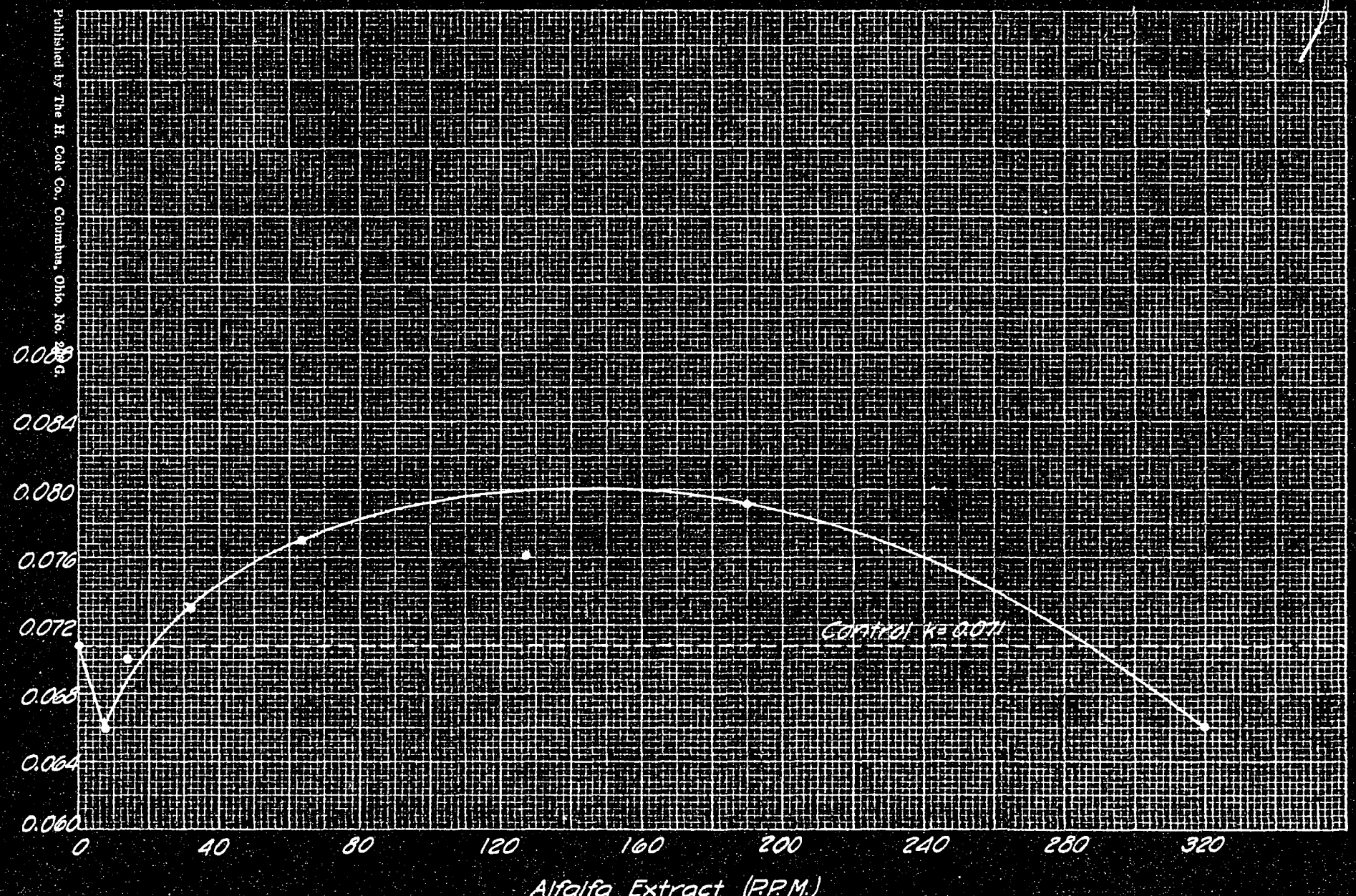


TABLE VII. THE OPTIMUM CONCENTRATIONS OF SOIL, MANURE AND ALFALFA EXTRACTS FOR THE GROWTH OF NON-STERILE LEMNA.

No.	p.p.m. stimulant added	k	Organic stimulant (The pH of extracts was kept constant at 4.8)
1	6	0.096	Soil extract
2	12	0.098	" "
3	24	0.099	" "
4	48	0.096	" "
5	72	0.095	" "
6	144	0.089	" "
7	200	0.087	" "
1	2	0.080	Manure extract
2	4	0.087	" "
3	5	0.090	" "
4	21	0.096	" "
5	100	0.101	" "
6	177	0.100	" "
7	270	0.095	" "
8	360	0.092	" "
1	8	0.066	x Alfalfa extract
2	16	0.070	" "
3	32	0.073	" "
4	64	0.077	" "
5	128	0.076	" "
6	192	0.079	" "
7	320	0.066	" "
1	---	0.071	Control

xMicro-organic growth attached to roots which could not be washed free.
Duration of experiment 2 weeks.

The data already given in this thesis on the effects of organic matter upon the growth constants of Lemna major show conclusively that many organic substances stimulate the growth of the green plant. Only in the cases of carrots and of manure suspension was there an indication of no stimulation at the concentrations used, but these organic substances were studied only over a limited range. While solutions of urea and asparagin were toxic to the growth of this plant, there was one concentration for each which slightly accelerated the growth. Every organic material investigated revealed an optimum concentration. This was found for urea and for asparagin compounds and for soil, manure and alfalfa extract. Among these, soil and manure extract were found to be stimulants in minute quantities as well as in larger amounts. Note that alfalfa extract depresses the growth constants of Lemna in very minute and in very large quantities, but for concentrations between these two extremes there is a decided stimulation. Soil extract, on the other hand, accelerates at all concentrations studied, while manure extract depresses only at 5 p.p.m. approximately. The irregularity of the optimum growth rate curve for alfalfa is perhaps due to an error in counting the plants for the fifth constant, or caused by some toxic substance falling into the solution in which the plants were growing. The fact that manure in the concentrations from 3 - 8 p.p.m. produces a depression in the growth rate of Lemna in one case, while in

another case such a depression is not evident in the quantities from 2-360 p.p.m., may be due to the use of different extracts in each instance.

It is now apparent that soil extract, ranging from minute to large quantities, stimulates the growth of the non-sterile green plant; that other organic substances act as stimulants in some concentrations and as depressants in others; that still other organic materials act as depressants in all concentrations studied; and that nearly all organic substances investigated tended to show an optimum point.

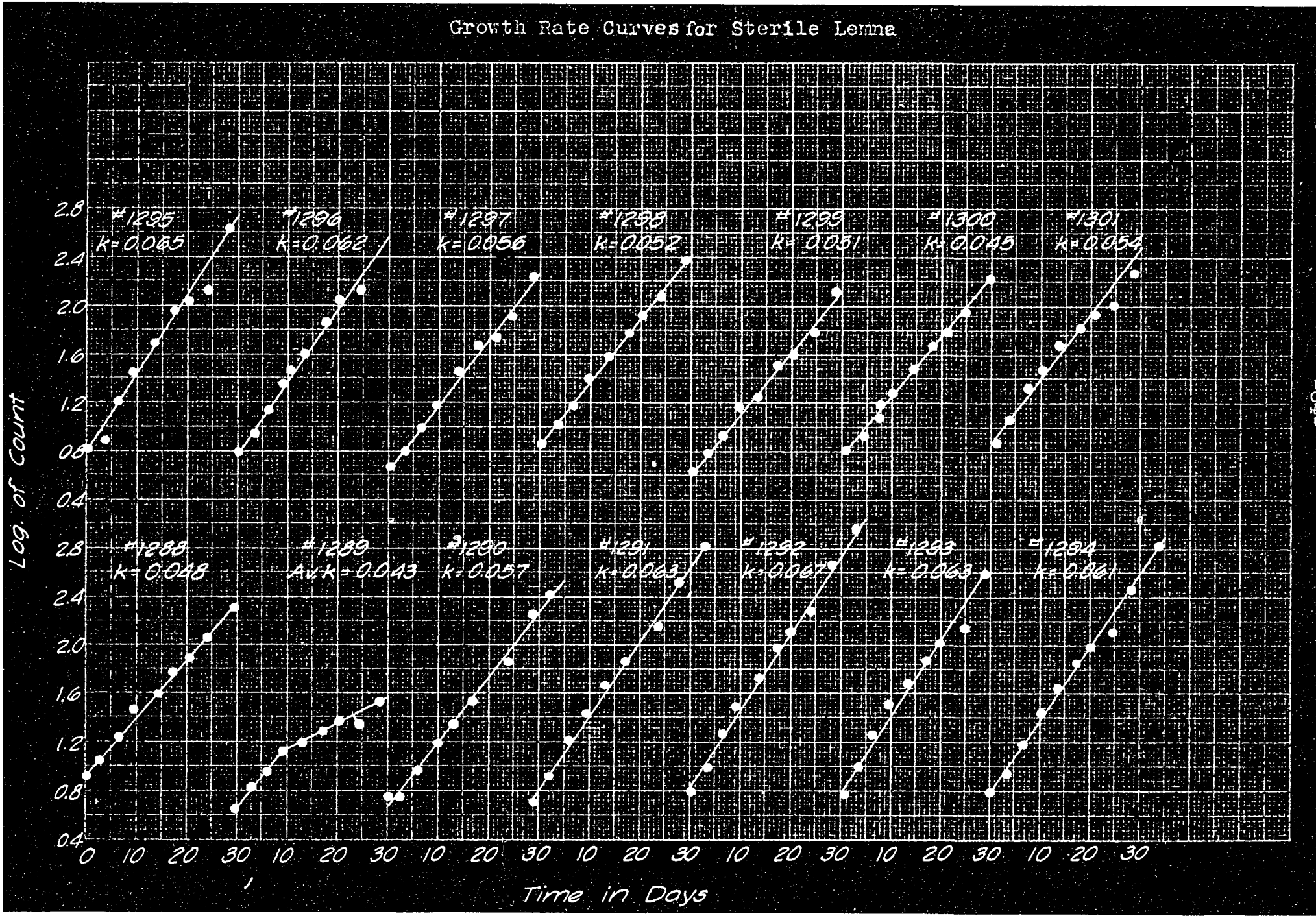
We next desired to investigate the effects of organic matter upon the growth of sterile Lemna major.

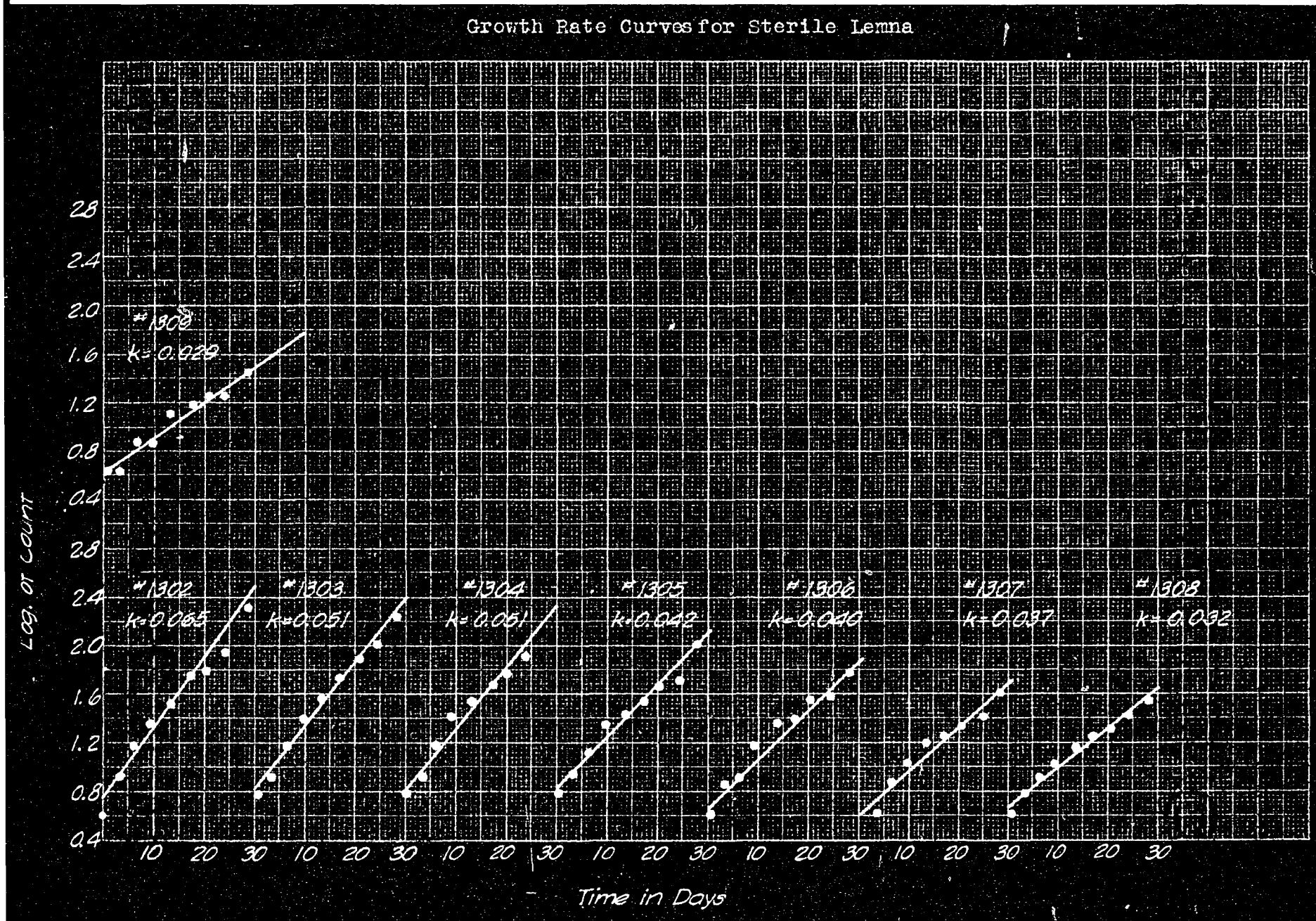
THE EFFECTS OF ORGANIC MATTER ON THE GROWTH RATE OF THE STERILE GREEN PLANTS.

In determining the effect of organic matter on the growth rate of sterile Lemna, we followed the same methods and technic as with the non-sterile plant, except that the solutions were sterilized, and the plants, both sterile and non-sterile, were grown in cotton stoppered 250 cc. Erlenmeyer flasks containing 100 cc. of modified solution 1 which had been sterilized as heretofore described. All transfers were made in a sterilized chamber already mentioned in the part of this thesis entitled "Methods and Technic". The initial pH was made up to the optimum as has been done previously for this solution. This procedure was followed in all subsequent experiments.

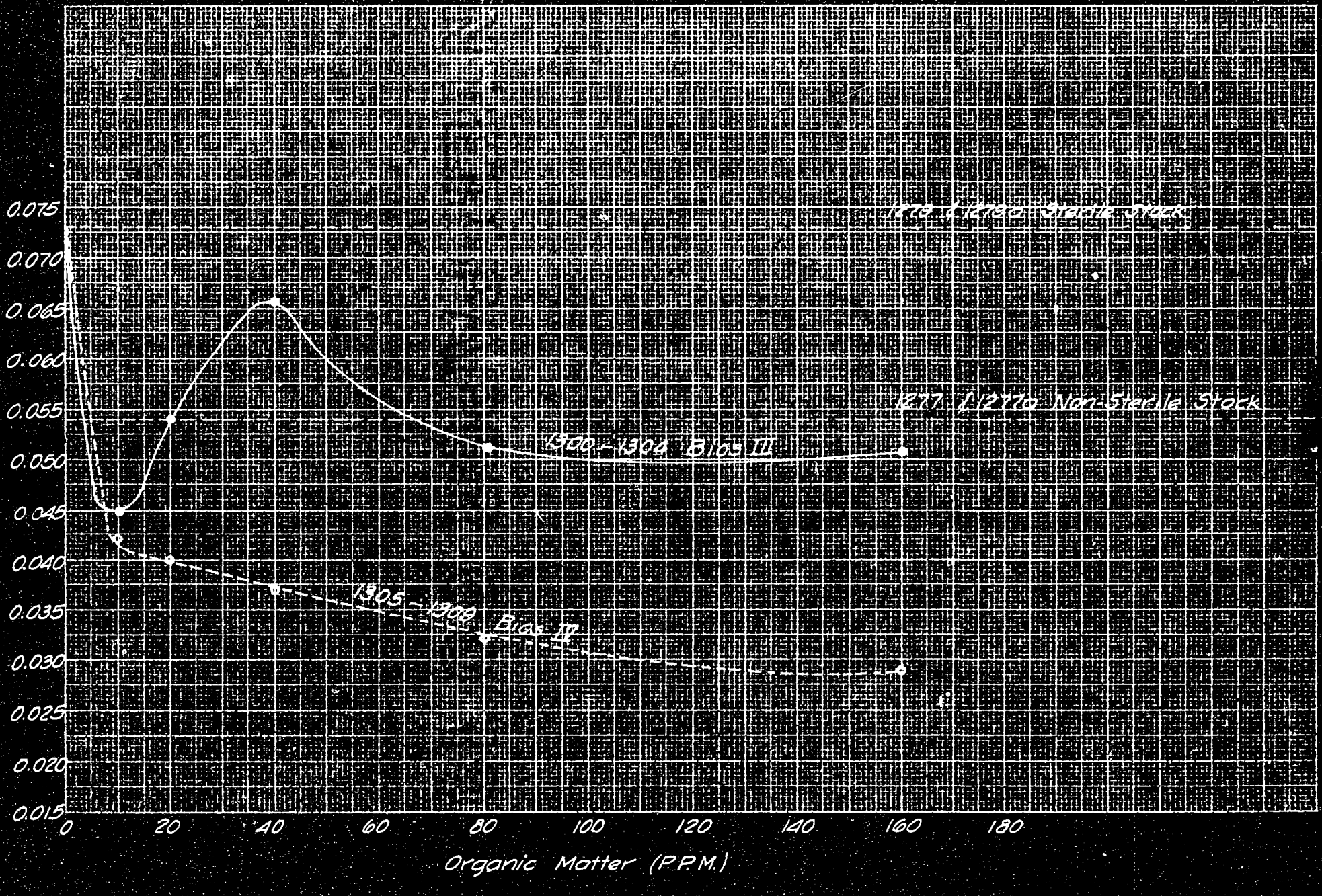
The accompanying graphs and table show the plan and the results of this experiment.

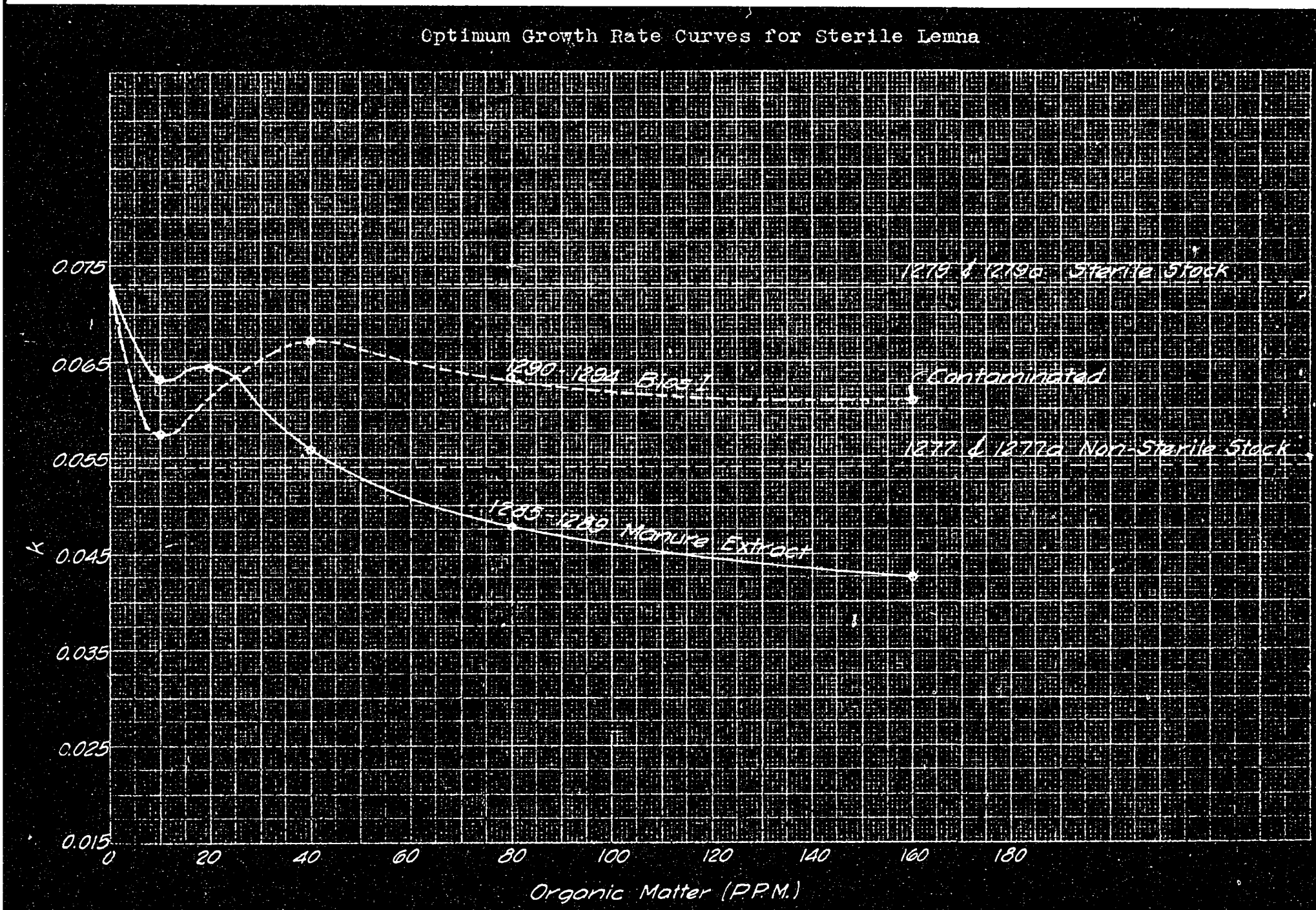
Growth Rate Curves for Sterile Lemna


Growth Rate Curves for Sterile Lemna
#1295 k=0.065
#1296 k=0.062
#1297 k=0056
#1298 k=0.052
#1299 k=0.051
#1300 k=0.045
#1301 k=0.054
#1288 k=0.048
#1289 Av k=0.043
#1290 k=0.057
#1291 k=0.063
#1292 k=0.067
#1293 k=0.063
#1294 k=0.061
Log. of Count
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0 10 20 30 10 20 30 10 20 30 10 20 30 10 20 30 10 20 30 10 20 30
Time in Days


Growth Rate Curves for Sterile Lemna
Log. of Count
Time in Days
#1302
k=0.065
#1303
k=0.051
#1304
k=0.051
#1305
k=0.042
#1306
k=0.040
#1307
k=0.037
#1308
k=0.032
#1309
k=0.029
2.8
2.4
2.0
1.6
1.2
0.8
0.4
10
20
30

Optimum Growth Rate Curves for Sterile Lemna




Optimum Growth Rate Curves for Sterile Lemna
0.075
0.065
0.055
0.045
0.035
0.025
0.015
K
0
20
40
60
80
100
120
140
160
180
Organic Matter (P.P.M.)
1279 & 1279a Sterile Stock
1290 1294 Blend
Contaminated
1277 & 1277a Non-Sterile Stock
1285 1286 Manure Extract

Form 23

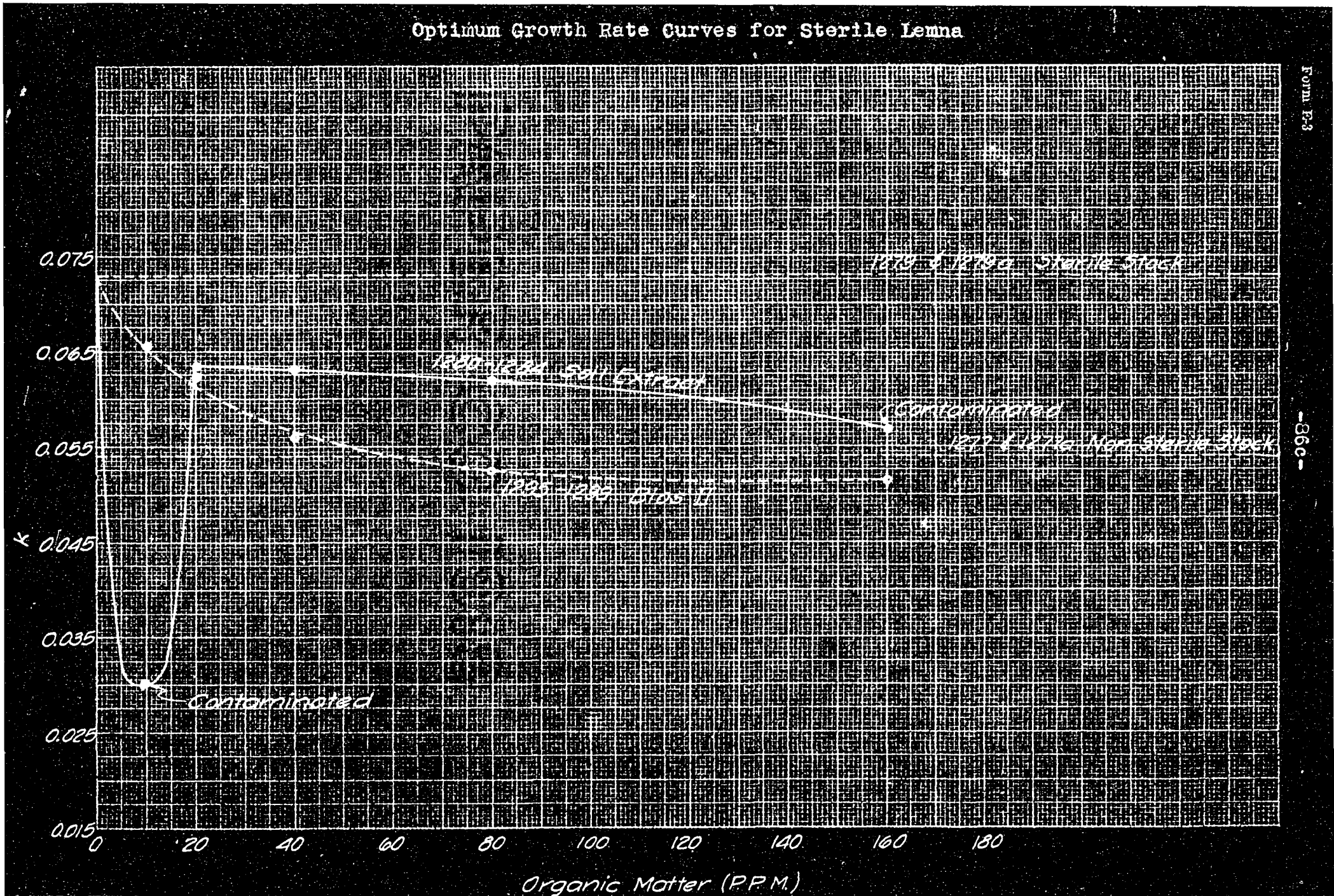

Optimum Growth Rate Curves for Sterile Lemna
0.075
0.065
0.055
0.045
0.035
0.025
0.015
K
0
20
40
60
80
100
120
140
160
180
Organic Matter (P.P.M.)
Contaminated
Contaminated

TABLE VIII. OPTIMUM CONCENTRATIONS OF VARIOUS ORGANIC EXTRACTS FOR THE GROWTH OF STERILE LEMNA

Number	Treatment	Plants from stock	k		Appearance at end	Sterility at end
1277	z + no addition--Sterilized	Non-sterile	0.053	54	a	n.s.
1277a	z + " " -- "	" "	0.055		a	n.s.
1278	z + " " --Not sterilized	Sterile	0.055	55	a	n.s.
1278a	z + " " -- " "	"	0.054		a	n.s.
1279	z + " " --Sterilized	"	0.072	73	a+1	s
1279a	z + " " -- "	"	0.074		a+1	s
1280	z + 10p.p.m.soil ext. "	"	0.030		a-10⊠	n.s. Δ
1281	z + 20 " " " "	"	0.063		a	s
1282	z + 40 " " " "	"	0.063		a	s
1283	z + 80 " " " "	"	0.062		a	s
1284	z +160 " " " "	"	0.057		a	n.s. Δ
1285	z + 10 " man.ext. "	"	0.063		a	s
1286	z + 20 " " " "	"	0.064		a	s
1287	z + 40 " " " "	"	0.055		a-1	s
1288	z + 80 " " " "	"	0.048		a-2	s
1289	z +160 " " " "	"	0.043		a-10	s
1290	z + 10 " BiosIext. " x	"	0.057		a-2	s
1291	z + 20 " " " "	"	0.063		a	s
1292	z + 40 " " " "	"	0.067		a	s
1293	z + 80 " " " "	"	0.063		a-1	s
1294	z +160 " " " "	"	0.061		a-2	n.s. Δ
1295	z + 10 " " II " " x	"	0.065		a-2	s
1296	z + 20 " " " "	"	0.062		a-2	s
1297	z + 40 " " " "	"	0.056		a-1	s
1298	z + 80 " " " "	"	0.052		a-2	s
1299	z +160 " " " "	"	0.051		a-2	s
1300	z + 10 " " III " "	"	0.045		a-2	s
1301	z + 20 " " " "	"	0.054		a-2	s
1302	z + 40 " " " "	"	0.065		a-1	s
1303	z + 80 " " " "	"	0.051		a-2	s
1304	z +160 " " " "	"	0.051		a-4	s
1305	z + 10 " " IV " "	"	0.042		a-5	s
1306	z + 20 " " " "	"	0.040		a-6	s
1307	z + 40 " " " "	"	0.037		a-7	s
1308	z + 80 " " " "	"	0.032		a-9	s
1309	z +160 " " " "	"	0.029		a-10	s

z = Modified soln. 1 Δ = Contamination. ⊠ = almost dead.
x These fractions were made according to Fulmer, Duecker and Nelson.
pH is held constant at 4.8. Duration of experiment 4 weeks.

An attempt was made to obtain optimum growth rate curves for sterile Lemna grown in solutions containing varying quantities of soil and manure extracts, and the bios fractions I, II, III and IV (22). A sterile and a non-sterile check were also run along with a sterile check whose solution was non-sterile.

Using the range from 10-160 p.p.m. of these organic extracts, we obtained curves which showed optimums, except those for bios II and IV. It is first noticed that the maximum points of these curves while in most cases lying above the line representing the non-sterile stock 1277 in no case touch the line representing the sterile stock 1279. This indicates, then, that the presence of sterile organic matter which has been used in this experiment depresses the growth rate of Lemna. This result is almost the converse of that obtained with the non-sterile plant. It appears, consequently, that organic matter, although an accelerator to the non-sterile plant, is toxic to the sterile one. Why some stimulants do not effect the two stock plants alike is a question which will b considered to a greater extent later in this thesis.

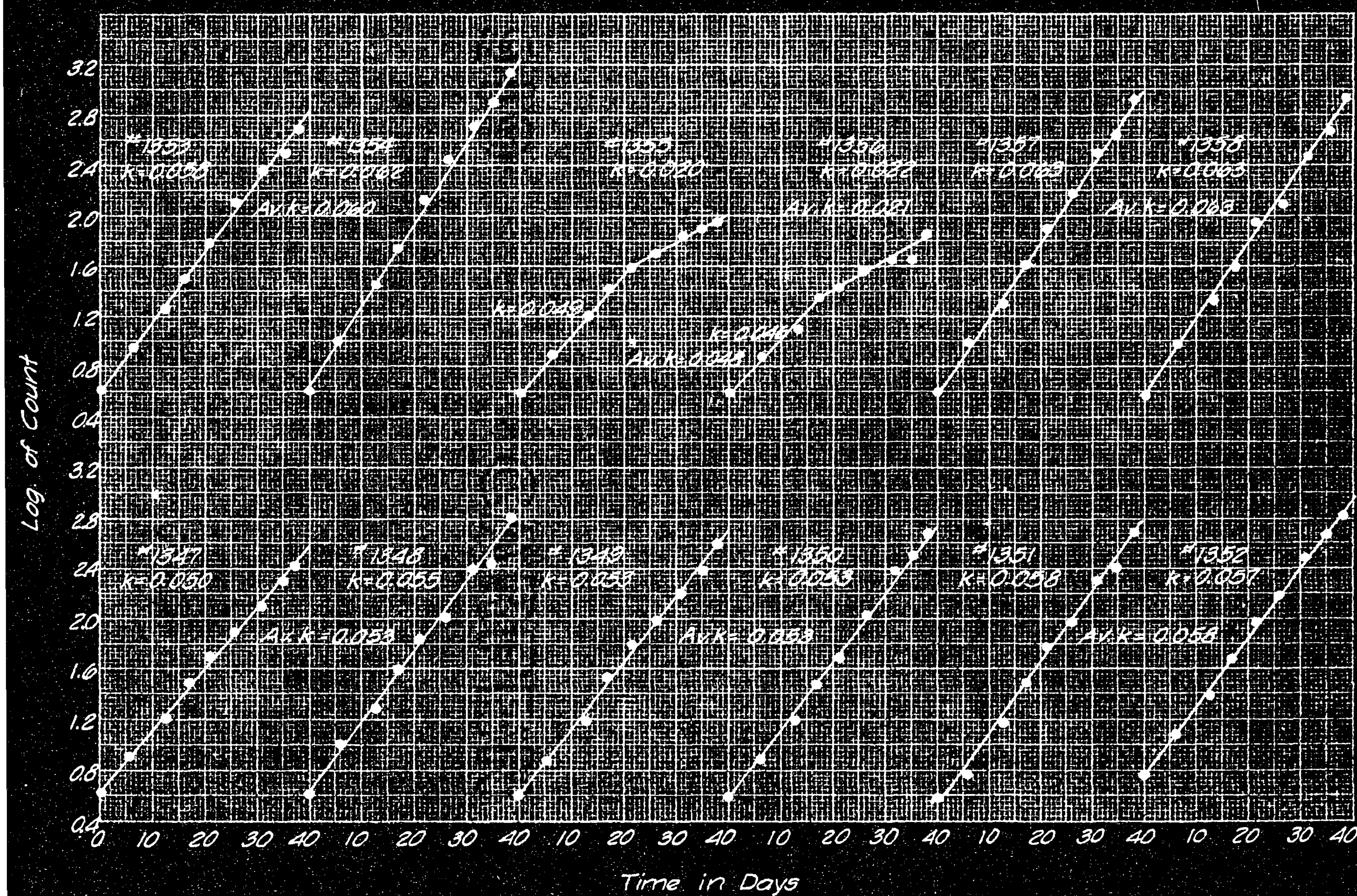
It is again observed from the graphs and table just preceding that the growth constants for the two stock plants are not alike: the one for the sterile plant is significantly higher than that for the non-sterile plant. It is therefore evident that Lemna can grow satisfactorily not only free of

all micro-organisms but also can continue to live and reproduce better when in a pure culture. In other words, it seems probable that micro-organisms are parasitic in their relation to Lemna, since the plant can grow better without them. Inasmuch as bacteria were seen in the plant by the aid of the oil immersion objective of a microscope, it seems likely that their presence in the interior of the frond and root of the plant is harmful to Lemna. The micro-organisms must live on the plant since there is no other source of organic matter present. By this theory, if the plant is free of these parasites, it should respond by an increase in growth rate.

The table shows further that the growth rate of sterile Lemna, upon being inoculated into a non-sterile modified solution 1, and becoming contaminated with bacteria, will soon be the same as that for the plant which was non-sterile originally.

It was next desired to determine what effects bacteria would produce when inoculated into the sterile solution containing both organic matter and the sterile plant. Soil and manure extracts were used as a source of organic matter. It was also desired to determine the effects of a dead culture upon the sterile plant. The following table and graph show the plan and results of this experiment.

Growth Rate Curves for Sterile Lemna



Form E-3

-90-

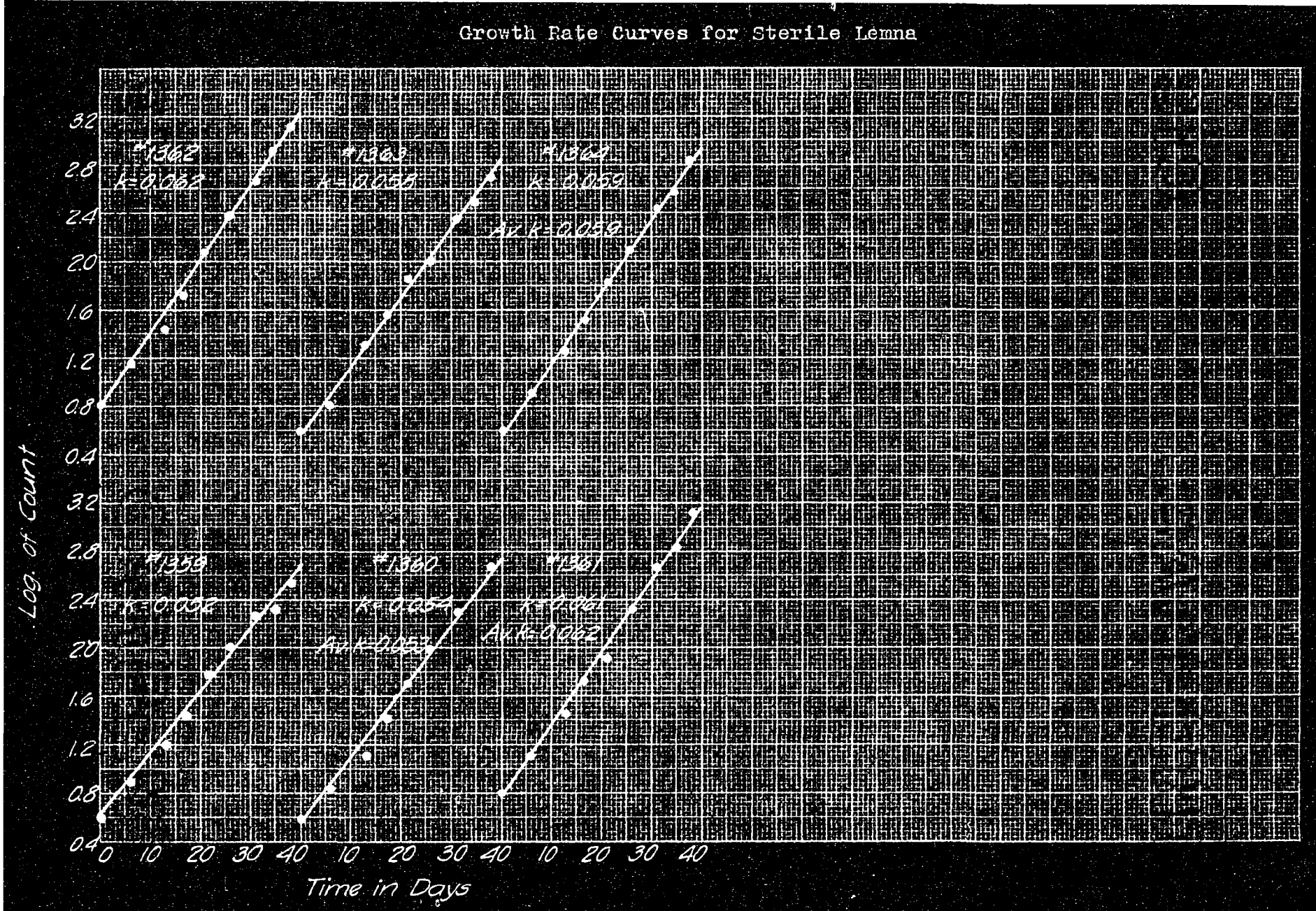

Growth Rate Curves for Sterile Lemna
Log. of Count
Time in Days
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0 10 20 30 40 10 20 30 40 10 20 30 40
r=1362
k=0.062
r=1363
k=0.065
r=1364
k=0.059
r=1359
k=0.065
r=1360
k=0.062
r=1361
k=0.062

TABLE IX. THE EFFECTS OF MICRO-ORGANISMS ON THE GROWTH OF LEMNA.

Number	Treatment	k	Appearance at end	Sterility at end	Plants from stock
1347	Mod.Sol.1 + 20p.p.m. soil ext.- Sterilized	0.050	a	s	Sterile
1348	" " + " " - "	0.055	a	s	"
1349	" " + 80 p.p.m.manure "	0.053	a	s	"
1350	" " + " "	0.053	a	s	"
1351	" " + 20 p.p.m. soil ext.-+ 1 drop BC[x]	0.058	a+1	n.s.	"
1352	" " + " " -+ 1 " "[x]	0.057	a+1	n.s.	"
1353	" " + 80 p.p.m.manure " -+ 1 " "[x]	0.058	a+1	n.s.	"
1354	" " + " " -+ 1 " "[x]	0.062	a+1	n.s.	"
1355	" " + 1 cc.[x] Bact. culture	0.035	dying	s	"
1356	" " + 1 cc.[x] " "	0.034	"	s	"
1357	" " + no addition	0.063	a+1	s	"
1358	" " + " "	0.062	a+1	s	"
1359	" " + " "	0.052	a	n.s.	non-sterile
1360	" " + " "	0.054	a	n.s.	" "
1361	" " + 20 p.p.m. soil ext.	0.061	a+1	n.s.	" "
1362	" " + 20 " " "	0.062	a+1	n.s.	" "
1363	" " + 80 p.p.m. manure ext.	0.058	a+1	n.s.	" "
1364	" " + 80 " " "	0.059	a+1	n.s.	" "

x Bacterial culture made by inoculating bacto-nutrient agar with a non-sterile plant, allowing to incubate, and making an aqueous suspension of the bacterial growth. The initial pH was not altered by the above additions. The final pH of 1355 and 1356 after 3 days was found to be 5.5. The initial pH was 4.8. Duration of experiment 5 weeks.

From the preceding table and graphs, it is observed that the sterile plants have a higher growth rate than the non-sterile, which result checks that previously cited. It is noted further that the constants for sterile plants grown in solutions containing organic extracts are smaller than those for the sterile check. The addition of bacteria to the soil and manure extracts increases the growth rates of the plants, but the constants do not reach those of plants grown in the sterile check. As was expected from previous experiments, the addition of manure and soil extracts to non-sterile Lemna increases the rate of growth over that shown for the non-sterile check, but even these non-sterile plants do not grow quite as fast as the sterile ones in the sterile inorganic solution. In fact, according to the results of this experiment, the sterile check exhibited the highest growth constant obtained in this experiment. Higher constants were anticipated, however, for the solutions containing organic matter which was inoculated from the non-sterile plants in one case, and from a bacterial culture in the other, than for the sterile check. Regardless of whether the micro-organisms used for inoculation were secured from a bacterial culture or from non-sterile plants, there was very little difference shown in the effect on the growth rates of the plants. The fact that a dead culture of micro-organisms was toxic to Lemna may be due to high pH as shown in Table IX.

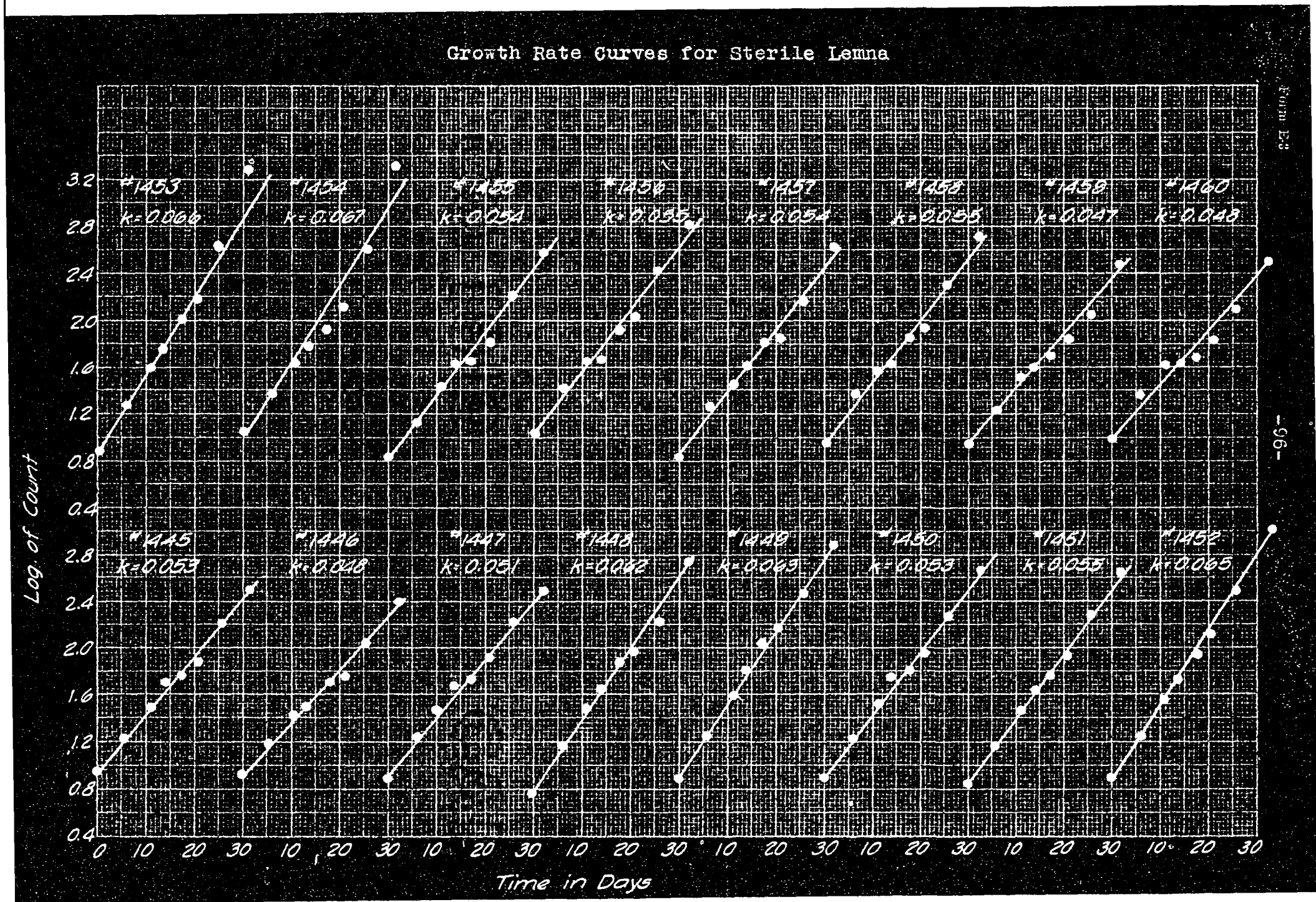
Thus far, there is an indication that some sterile or-

ganic matter depresses the growth of sterile Lemna, and while this growth constant is increased by the addition of bacteria, it does not exceed the constant given by the sterile check. There is an indication that dead bacteria depress the growth of the green plant, and there is further proof that sterile Lemna grows faster and looks better than the non-sterile.

Some of these debatable points will be further investigated and the results given later in this manuscript. The fact that the sterile organic matter was inoculated with bacteria from an old culture, and the fact that the organic matter used was an old extract, might be responsible for the depression of the growth rate of Lemna. The presence of sterile organic substances might account for the inability of the plants to equal the rate of those in the sterile stock, when inoculated with micro-organisms. Consequently, instead of using the aqueous suspension of bacteria, in future experiments, a fresh and virile strain will be used for inoculation when the plants are changed each time; and a fresh manure extract will be made and used instead of the old one.

In the ensuing experiment, we attempted to investigate further some of the questionable points which have arisen thus far in our work on the sterile plant. Our bacterial culture was fresh at all times and the manure extract used was freshly made up. We also attempted to determine the effects of pure compounds of urea, acetamide, and creatinine upon the sterile plant under sterile and non-sterile conditions, ar-

bitrarily selecting 200 p.p.m. of each as the concentration to use. The table and graphs to follow will illustrate the plan and give the results of this experiment.


Growth Rate Curves for Sterile Lemna
Log. of Count
Time in Days
#1453 k=0.066
#1454 k=0.067
#1455 k=0.054
#1456 k=0.055
#1457 k=0.054
#1458 k=0.055
#1459 k=0.047
#1460 k=0.048
#1445 k=0.053
#1446 k=0.048
#1447 k=0.051
#1448 k=0.062
#1449 k=0.063
#1450 k=0.053
#1451 k=0.055
#1452 k=0.065

Growth Rate Curves for Sterile Lemna

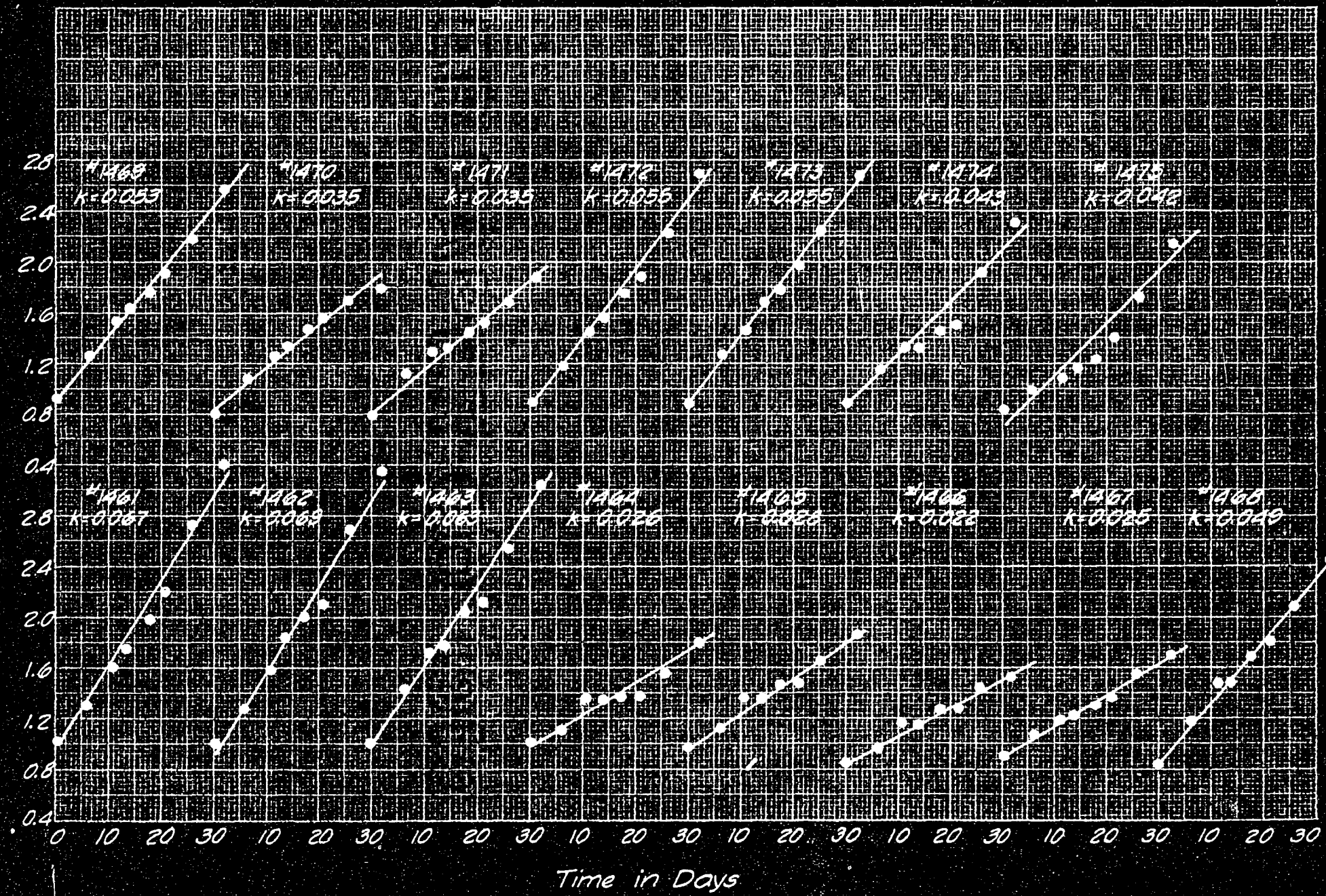


TABLE X. THE EFFECTS OF MICRO-ORGANISMS ON THE GROWTH OF LEMNA.

Number	Treatment	Plants from stock	k		App. at end	Sterility at end
1445	z + no addition Sterilized + no addition	Sterile	0.053	52	a	s
1447	z + " " " + " "	"	0.051		a	s
1448	z + " " " + 80 p.p.m. non-sterile manure extract	"	0.062	63	a+1	n.s.
1449	z + no addition Sterilized + " " " sterile manure extract	"	0.063		a+1	n.s.
1450	z + 80 p.p.m. manure ext. Sterilized + no add.	"	0.053	54	a+1	Contaminated
1451	z + " " " " " + " "	"	0.054		a+1	"
1452	z + " " " " " +1 drop 1476	"	0.065	66	a+1	n.s.
1453	z + " " " " " +1 " "	"	0.066		a+1	n.s.
1454	z + " " " " " +1 " "	"	0.067		a+1	n.s.
1455	z + no addition Sterilized + 1 drop 1476	"	0.054		a+1	n.s.
1456	z + " " " + 1 " "	"	0.055	55	a+1	n.s.
1457	z + " " " + 1 " 1477	"	0.054		a+1	n.s.
1458	z + " " " + 1 " "	"	0.055	55	a+1	n.s.
1459	z + " " " + no addition	Non-sterile	0.047		a-1	n.s.
1460	z + " " " + " "	" "	0.048	48	a-1	n.s.
1461	z + 80 p.p.m. manure ext. Sterilized + 1 drop 1476	Sterile	0.067	67	a+1	n.s.
1462	z + " " " " " + no add.	Non-sterile	0.069	69	a+1	n.s.
1463	z + no addition Sterilized + 80 p.p.m. non-sterile manure ext.	" "	0.063	63	a+1	n.s.
1464	z + 200 p.p.m. urea " + no addition	Sterile	0.026		a-6	s
1465	z + " " " " + " "	"	0.028	27	a-6	Contaminated
1466	z + " " " " + 1 drop 1476	"	0.022		a-6	n.s.
1467	z + " " " " + 1 " "	"	0.025	24	a-6	n.s.
1468	z + " " acetamide Sterilized + no add.	"	0.049		a+1	s
1469	z + " " " " + " "	"	0.053	51	a+1	s

TABLE X (cont.)

Number		Plants from Stock	k		App. at end	Sterility at end
1470	z + 200 p.p.m. acetamide Sterilized + 1 drop 1476	Sterile	0.035	35	a-6	n.s.
1471	z + " " " " + 1 " "	"	0.035		a-6	n.s.
1472	z + " " creatinine " + no add.	"	0.055	55	a+1	Contaminated
1473	z + " " " " + " "	"	0.055		a+1	s
1474	z + " " " " + 1 drop 1476	"	0.043	43	a	n.s.
1475	z + " " " " + 1 " "	"	0.042		a	n.s.

1476 Bouillon is inoculated with non-sterile plants and allowed to incubate for 3 days.
1477 Bouillon same as 1476 but sterile.
1478 Solution 1 in which plants have been growing.
The pH of modified solution 1 is not altered by the above additions.

z = modified solution 1
pH is held constant at 4.8.
Duration of experiment 5 weeks.

A review of the preceding table shows that the rate of reproduction of the sterile plant is greater than that of the non-sterile plant grown in the sterilized medium. This confirms the results obtained twice before. The table also shows in opposition to the results in the preceding experiments, that when Lemna is grown in the medium containing manure extract which is inoculated with micro-organisms, the rate of reproduction is increased over that shown by the sterile plants in the sterile medium.

The sources of micro-organisms for the inoculation of the manure extract used in this experiment are as follows:

1448)
1449) Modified Sol. 1 sterilized + manure - K = 0.063 - From the manure.

1451)
1452) Modified Sol. 1 + manure sterilized + 1 drop 1476
1453) -K = 0.066 - From non-sterile plants.

1461 Modified Sol. 1 + manure sterilized + 1 cc. 1478- K = 0.067 - From non-sterile solution.

Sterilizing the manure does not seem to injure its stimulating properties toward Lemna.

1462 Modified Sol. 1 + manure extract sterilized + non-sterile plants - k = 0.069 - From non-sterile plants.

1463 Modified Sol. 1 sterilized + manure extract + non-sterile plants - k = 0.063 - From manure and non-sterile plants.

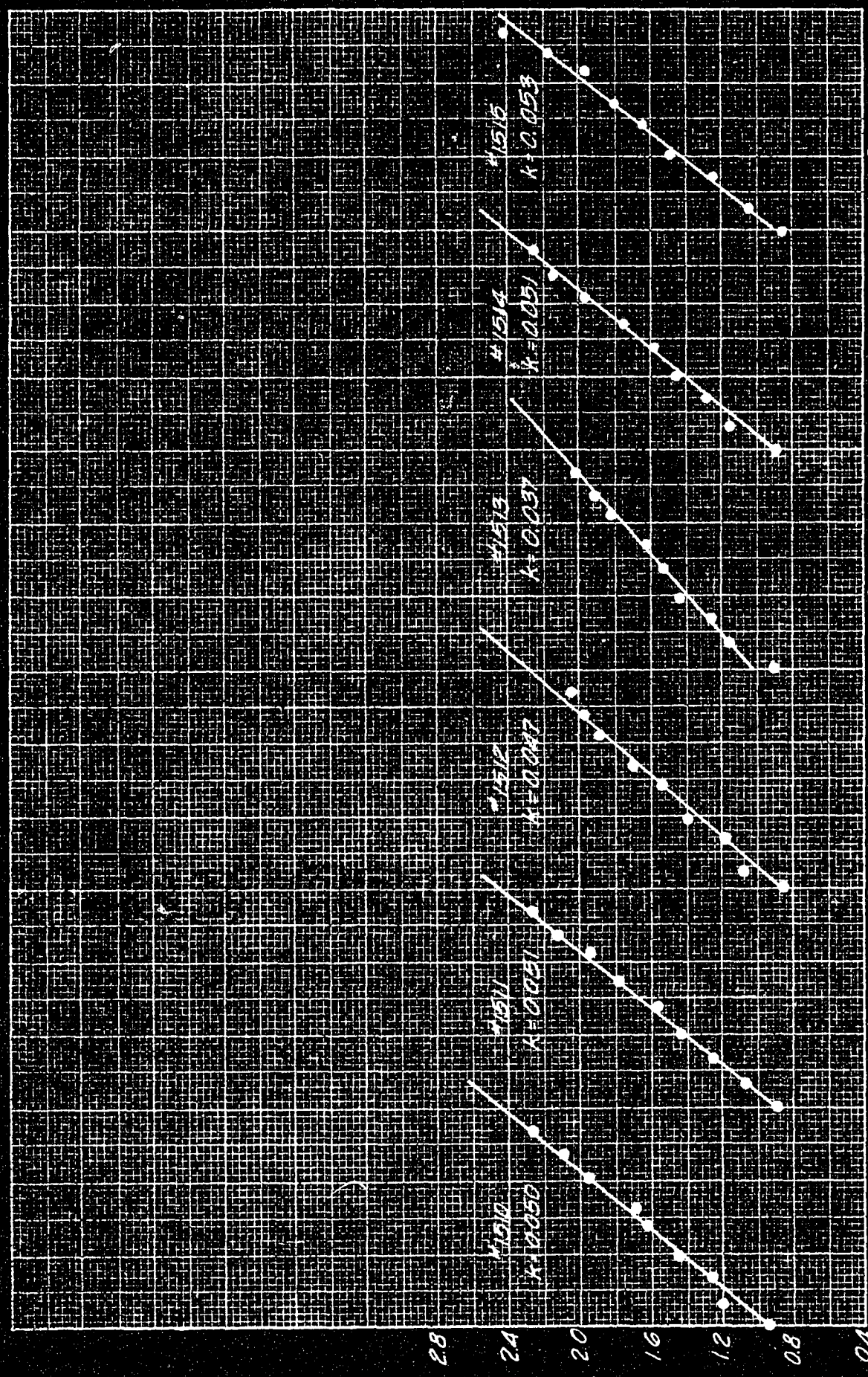
Regardless of the source of the inoculation of the organic matter, then, the rate of reproduction of the plant is greater than that of the sterile check. It is noticed that the constants for sterile and non-sterile plants grown in so-

lutions containing inoculated organic matter are about the same, as one would expect. Number 1463 shows an increase in the rate of reproduction over that of non-sterile plants grown in solutions containing non-sterile manure extract. This checks previous results. In this experiment, sterile manure has no apparent effect on the growth rate of Lemna. No effect on rate of reproduction was noticed for 1 drop of sterile and non-sterile bouillon. The bouillon was found contaminated at the end. Bacteria from the non-sterile plants were kept virile by inoculating a fresh tube of sterile bouillon each time the plants were changed to fresh solutions. To inoculate sterile manure with these micro-organisms was added 1 drop of the bouillon which had been previously inoculated with a non-sterile plant and incubated 3 days. Since it was desired to ascertain whether the bouillon or the bacterial products would effect the growth of Lemna, two solutions of sterile and two of non-sterile bouillon were employed. It was found that inoculating the solutions containing the pure organic compounds injured the plants, the sterile ones doing better than the non-sterile.

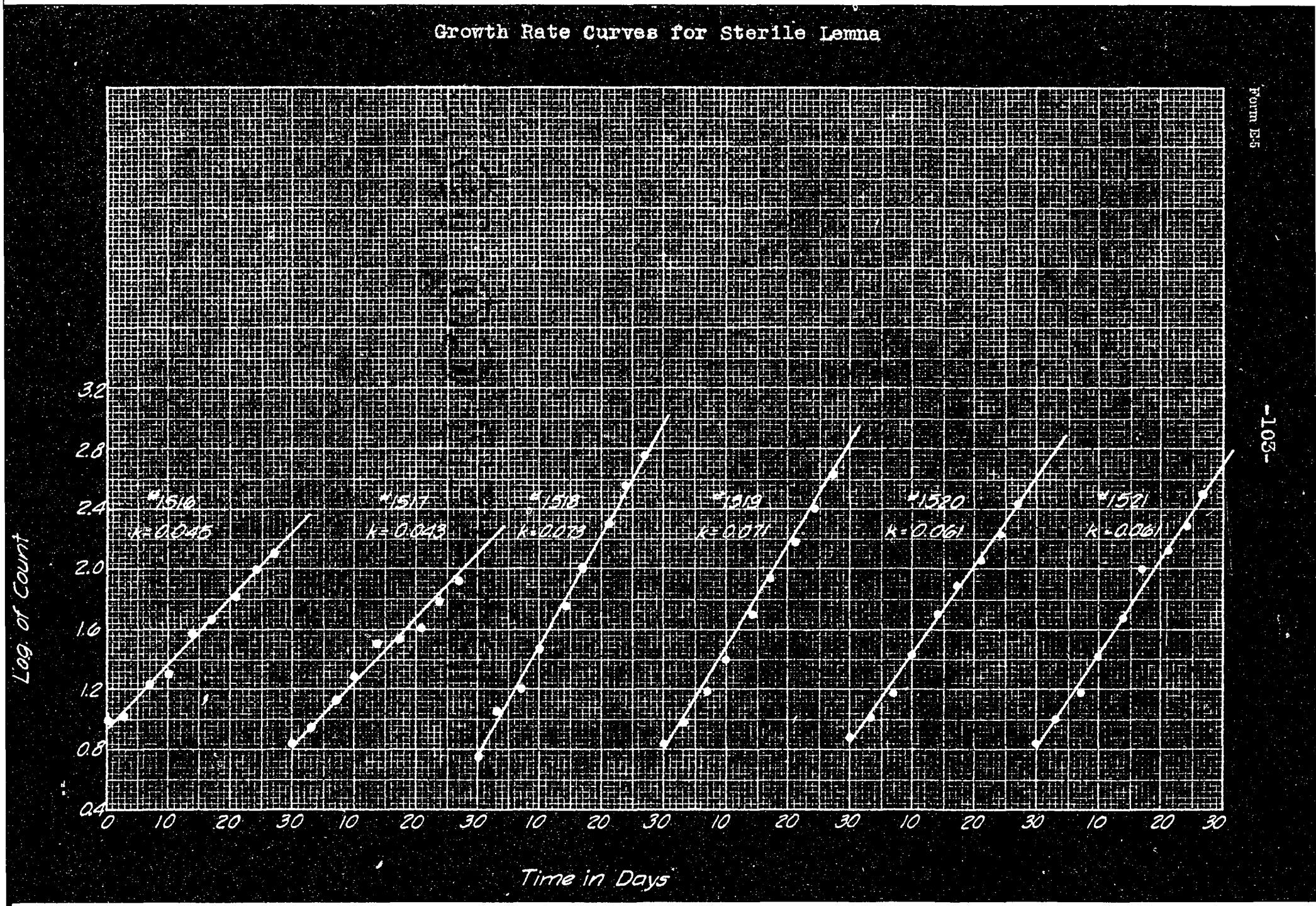
The experiment just described was repeated. In so doing, it was hoped to gain better technic, and to obtain more definite and significant results on the effects of organic matter upon the rate of reproduction of the sterile plant. It was also desired to determine the effects of pure cultures

of bacteria upon the growth of sterile Lemna in organic solutions. The outline and results of the experiment are found in the following table and graphs.

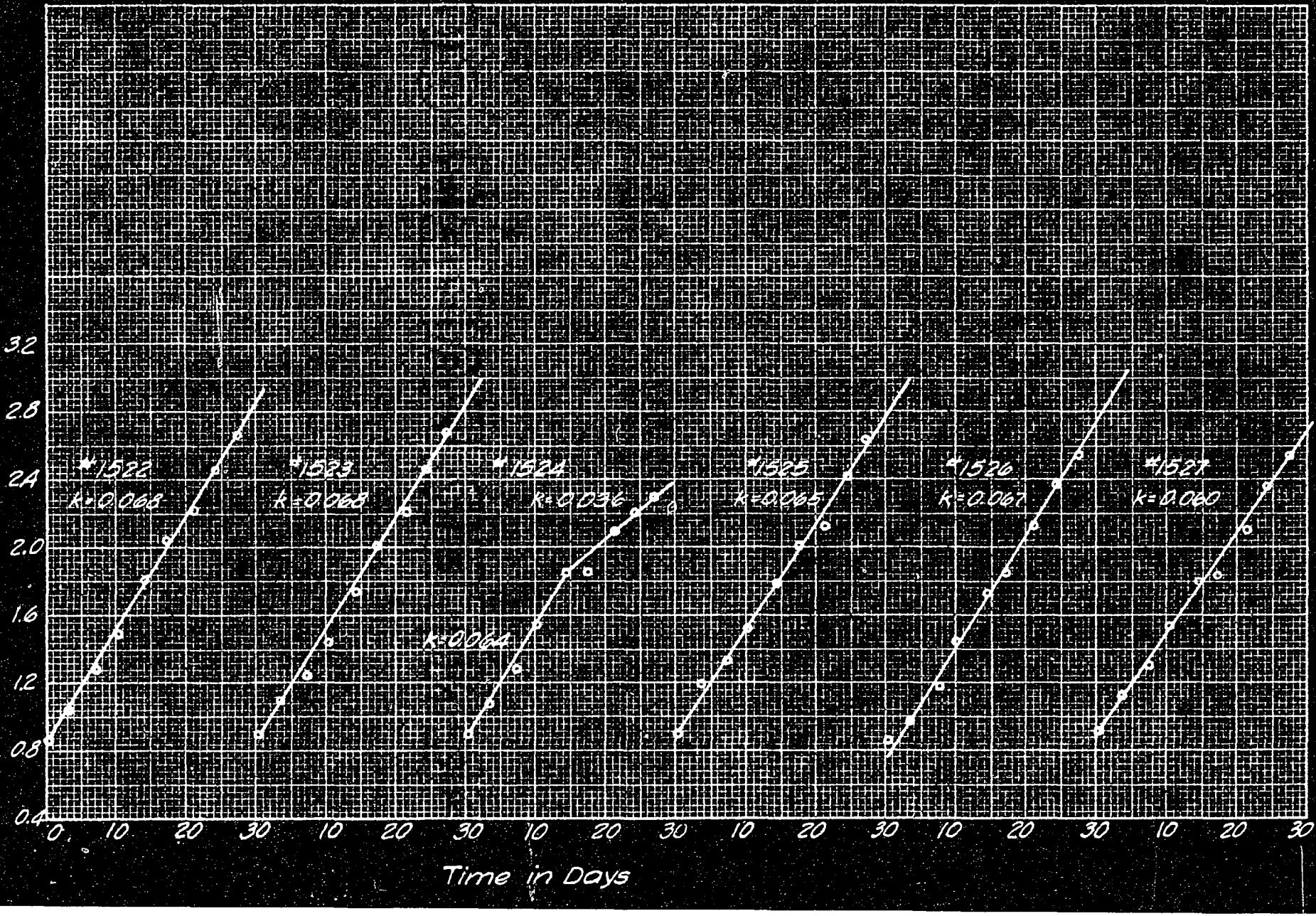
Form E-5


Growth Rate Curves for Sterile Lemna
#1516
k=0.053
#1514
k=0.051
#1513
k=0.037
#1512
#1511
Log. of Count
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0
10
20
30
Time in Days

Form E-5


Growth Rate Curves for Sterile Lemna
Log. of Count
Time in Days
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0
10
20
30
#1516
k=0.045
#1517
k=0.043
#1518
k=0.073
#1519
k=0.071
#1520
k=0.061
#1521
k=0.061

Growth Rate Curves for Sterile Lemna



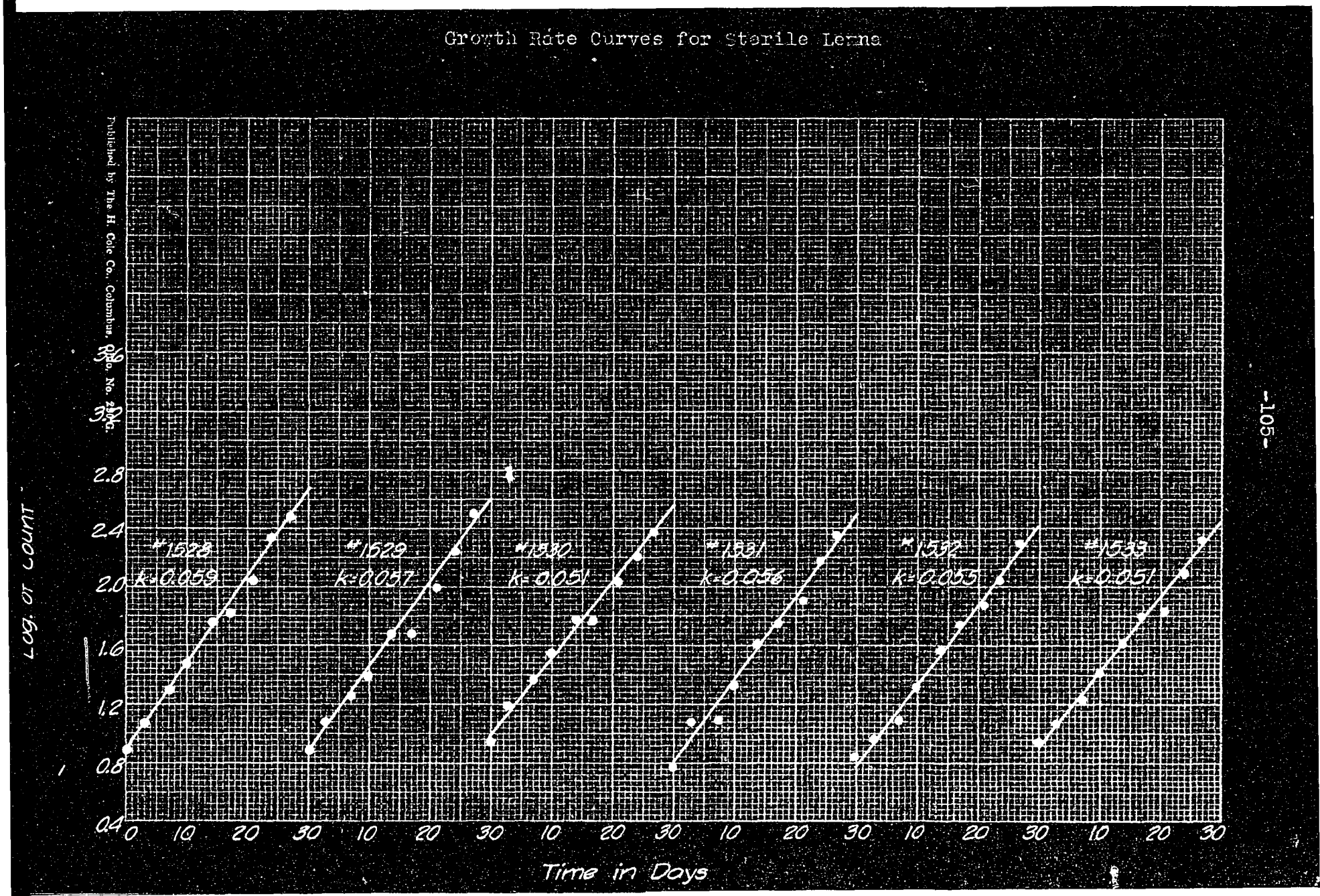


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TABLE XI. THE EFFECT OF MICRO-ORGANISMS ON THE GROWTH OF LEMNA

Number	Treatment	Plants from Stock	Inoculation	k		Sterility at end	App. at end
1510	z + no addition	Sterilized	Sterile	No addition	0.050	51	s	a
1511	z + " "	"	"	" "	0.051		s	a
1512	z + " "	"	"	x x	0.047		n.s.	a-2
1513	z + y	"	"	No addition	0.037		s	a-3
1514	z + manure ext. (80 p.p.m.)	"	"	" "	0.051	52	s	a
1515	z + " " (80 ")	"	"	" "	0.053		s	a
1516	z + alfalfa ext. (130 p.p.m.)	"	"	" "	0.045	44	s	a-6
1517	z + " " (130 ")	"	"	" "	0.043		s	a-6
1518	z + manure ext. (80 p.p.m.)	"	"	x x	0.073	72	n.s.	a+2
1519	z + " " (80 p.p.m.)	"	"	x x	0.071		n.s.	a+2
1520	z + alfalfa ext. (130 p.p.m.)	"	"	x x	0.061	61	n.s.	a+1
1521	z + " " (130 ")	"	"	x x	0.061		n.s.	a+1
1522	z + manure ext. (80 p.p.m.)	"	Non-sterile		0.068	68	n.s.	a+1
1523	z + " " (80 ")	"	" "		0.068		n.s.	a+1
1524	z + " " (80 ")	"	Sterile	Bact. coli (1)	0.050 (av.)		n.s.	a-5
1525	z + " " (80 ")	"	"	Erythro bacillus pro-digiosus (2)	0.065		n.s.	a+1
1526	z + " " (80 ")	"	"	B. subtilis (3)	0.067		n.s.	a+1
1527	z + " " (80 ")	"	"	Staph. aureus (4)	0.060		n.s.	a+1
1528	z + " " (80 ")	"	"	Cl. Welchii (5)	0.059		n.s.	a+1
1529	z + " " (80 ")	"	"	Bact. aero-genes (6)	0.057		n.s.	a+1
1530	z + " " (80 ")	"	"	Cl. sporo-genes (7)	0.051		n.s.	a+1
1531	z + " " (80 ")	"	"	Ps. fluores-escens lique-faciens (8)	0.056		n.s.	a+1
1532	z + " " (80 ")	"	"	No. 6 from Smith (9)	0.055		n.s.	a+1
1533	z + " " (80 ")	"	"	No.11 from Smith (10)	0.051		n.s.	a+1

z = modified solution 1 . pH held constant at 4.8
pH for this solution not changed for this addition
x Bouillon inoculated with organism from non-sterile plant from soln. 820.
y Sterile bouillon - 1 drop used for inoculation.
Duration of experiment - 4 weeks

An examination of the results of Table XI shows that sterile manure extract has no significant effect on sterile Lemna. This checks the results in Table X. Previous results with the sterile plant have shown, however, that all the organic substances used including manure extract, depressed the rate of reproduction. In no case thus far have we observed any significant stimulation of sterile Lemna with sterile organic matter; instead, in many cases a depression has been noted. An increased rate of reproduction over that of the sterile check has been brought about by inoculating the organic solution with micro-organisms. It is obvious that while the results with sterile manure check in Tables VIII and IX and again in X and XI, they do not check in all four tables. No attempt is made to explain this discrepancy except that in the last two experiments, in which sterile manure had no effect on sterile Lemna, a fresh extract was used. In the first experiments, on the other hand, in which the growth rate of the sterile plant was depressed slightly, an old extract was employed. It seems quite possible that from the same supply of dry manure two samples may be obtained which will have quite different effects upon sterile Lemna, inasmuch as each sample used may contain different quantities of toxic substances. It will be remembered that the four fractions of the aqueous extract of alfalfa, and other organic materials were somewhat toxic to the sterile green plant, and in Table XI it is noticed that alfalfa extract depresses the rate of

reproduction of this plant when grown under sterile conditions. Even though manure extract decreased the rate of growth in two experiments, the appearance of the plants was equally as good as that of the sterile check. This was true of manure in all experiments with sterile Lemna, as well as soil extract and some of the alfalfa fractions when their optimum concentrations were used. It seems, then, that this single discrepancy which is shown in these last four experiments is not unduly significant, but the fact that no sterile organic substance used increased the growth constant of sterile Lemna over that of the sterile check is very significant and decidedly interesting. This would indicate that the plant can not utilize the complex organic compound contained in these extracts unless bacteria are present to change them into available forms, if the rate of reproduction is used as a criterion for this utilization. Had we investigated, however, a larger number of extracts and compounds, it is possible that some organic substance could have been discovered which would have stimulated the growth of sterile Lemna.

According to Martin and Mason (28), who investigated the influence of cane sugar on the conductivity of KCl, HCl and KOH, the conductivity of the inorganic solution is lowered when sugar is added to it. The conductivity of a solution and its ion and molecular activity are directly proportional, so it would seem that the presence of organic substances in a solution which are not in a state of availability would decrease the ion and molecular activity of that solution, and

this, in turn perhaps, would decrease the availability of the inorganic nutrients and finally would decrease the growth rate and eventually harms the plant. The plant must struggle harder to obtain its inorganic nutrients in a sterile organic solution than in a solution in which micro-organisms act on the organic matter to make it available.

In Science, March 7, 1930, Dr. N. A. Clark (17) who is directing this work, announced this investigation with the sterile plant. He reported the results of the first two experiments and stated that the addition of optimum concentrations of sterile organic extracts of soil and manure to the sterile inorganic medium decreased the rate of growth of the sterile plants as compared to those of the sterile check. We have already mentioned that, though this is true for the first experiments, the last results distinctly show that the sterile manure extract used later showed no effects on sterile Lemna.

Judging from the results that we have obtained thus far, it is evident that the micro-organisms present on non-sterile Lemna are harmful to the plant. These organisms are undoubtedly parasitic in their relation to the plant and not symbiotic, and when organic matter is added either to the sterile plant inoculated with bacteria or to the non-sterile plant, it is possible that the activity of the parasites is changed from the less available organic matter of the plant to the more available organic material added. This change in

activity would benefit the plant, as would also the products formed from the action of the organisms on the organic material added. No attempt was made to determine the effects of specific organisms on the sterile plant; the problem was merely to determine the effects of inoculating the non-sterile solution with sterile Lemna, in which case we have already seen that the growth constant fell back to that of the non-sterile plant. This is shown in Table VIII, and experiment number 1278.

It was observed from the results in Table XI that while sterile alfalfa extract depresses the growth rate of sterile Lemna, this rate was increased over that for the sterile check when this organic solution was inoculated with bacteria from the non-sterile plant. Various species of bacteria when inoculated into the sterile inorganic medium containing manure and sterile Lemna brought about an increase of the reproduction rate of the plant. Bacillus subtilis and Bacillus prodigiosus gave the greatest increase. No organisms other than those listed in Table XI were studied nor were there investigated other relations between the plant, organic matter and bacteria.

The effects of Carrington loam soil on the growth of non-sterile Lemna have already been given and described previously in this thesis. It is now desired to determine the effects of the same soil on the sterile plant, using the same method and technic employed with the non-sterile plant, except that

250 cc. beakers were used for growing and the non-sterile technic was used for changing the non-sterile Lemna.

The following table and graphs show the effects of sterile and of non-sterile Carrington loam soil on the reproduction rate of the green plant. The results show that the sterile plants grown in the sterile aqueous suspension of Carrington loam soon become unhealthy and began to die, while their growth rate was slightly smaller than that for the sterile check. These results are similar to those obtained for the effects of other sterile organic substances on the growth of the sterile plant. It will be remembered, however, that an aqueous suspension of non-sterile Carrington loam soil in concentrations of 80 p.p.m. or more of dissolved matter increased the growth rate of Lemna over that of the non-sterile check. Another sample of Carrington loam soil, equal in size and treated the same as the above sterile sample, but inoculated at each change of the plants with a grain of the non-sterile soil, greatly increased the growth rate of sterile Lemna. These results are similar to others obtained with the effects of non-sterile organic matter on the growth of the plant.

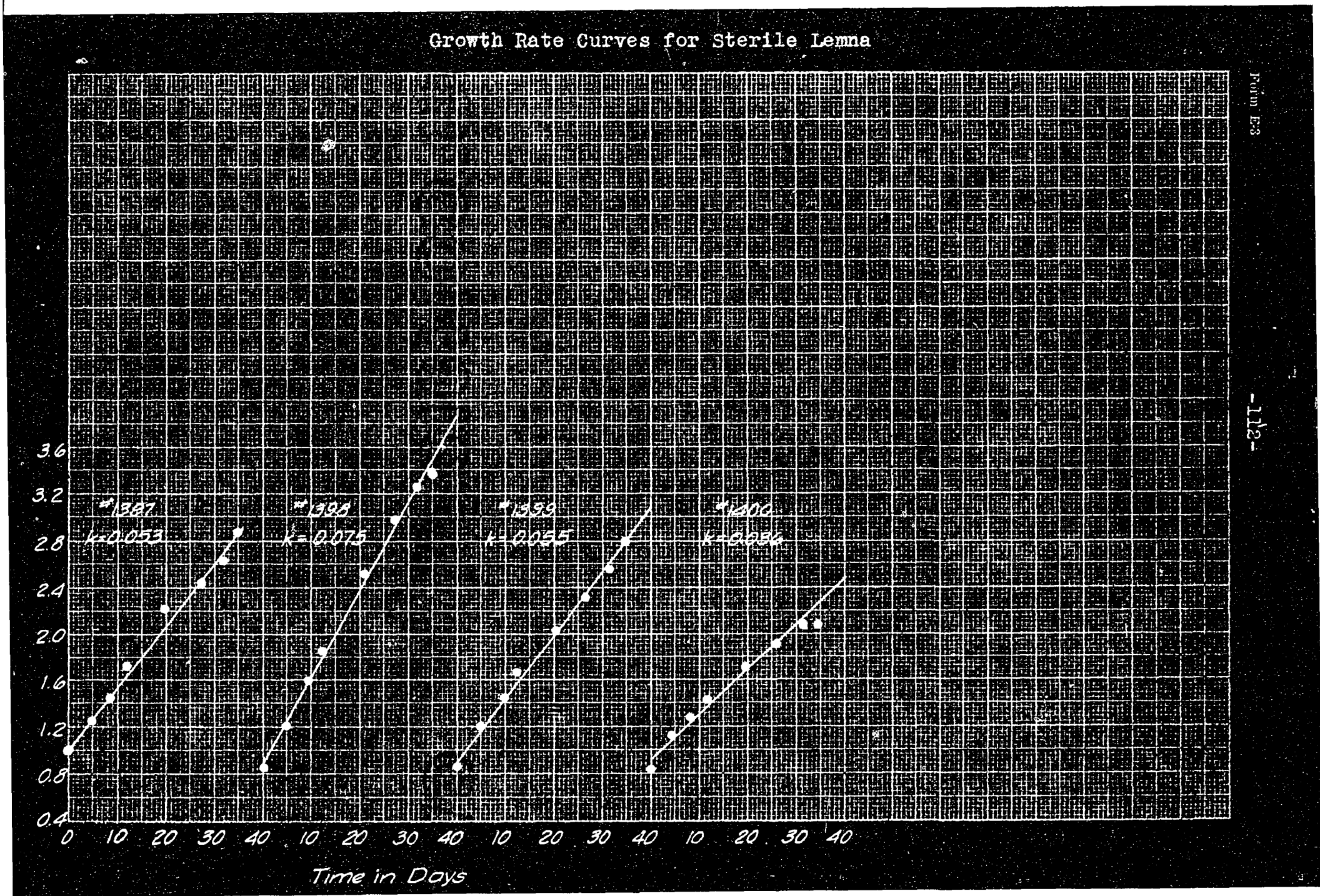

Growth Rate Curves for Sterile Lemna
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
k=0.053
k=0.075
k=0.055
0 10 20 30 40 10 20 30 40 10 20 30 40 10 20 30 40
Time in Days

TABLE XII. THE EFFECTS OF MICRO-ORGANISMS ON THE GROWTH OF LEMNA.

Number	Treatment	Plant from stock	k	pH	Appearance at end	Sterility at end
1397	10 grs. Carrington loam + 100cc. Con. H_2O sterilized	Sterile	0.053	6.90	a-5	s
1398	10 grs. Carrington loam + 100cc. Con. H_2O sterilized + bacteria[a]	"	0.075	6.36	a+1	n.s.
1399	Modified solution 1 sterilized	"	0.055	4.80	a	s
1400	" " " "	Non-sterile	0.036	4.80	a-2	n.s.

a Bacteria added by adding a very small quantity of non-sterile Carrington loam.

Duration of experiment 5 weeks.

THE GROWTH OF STERILE AND NON-STERILE LEMNA IN MODIFIED SOLUTION 1 NOT CHANGED.

It is noticed in the table and graphs following that the non-sterile plant is injured less and has a better appearance than the sterile one when both are grown continuously in the inorganic medium of identical composition. At the end of each experiment, the sterile plants were all looking badly and were showing the effects of continuous contact with the solution while the opposite was true with the non-sterile plants. No effort was made to keep the pH constant during each experiment, and perhaps the plants would have looked better and would have grown longer had this been done. No explanation is offered for the different appearance of the sterile and non-sterile plant. Though both of these plants should be changed to fresh solutions twice a week, they will reproduce and look quite healthy if not changed as often. Their optimum growth rate and best appearance, however, were obtained when changed about twice a week.

The results of 1495 in Table XIII confirm those obtained earlier in 1355 and 1356 in Table IX. These results at first indicated that a dead culture of organisms obtained from the non-sterile plant was toxic to the sterile plant grown in the sterile inorganic medium. Since the pH of this medium after three days was 5.5, we believed that, possibly, the toxic nature of the solution was due not to the organisms but to the high pH. The results of 1495 in Table XIII show that the

toxic nature of this medium was undoubtedly caused by the dead bacteria and not to the high pH, inasmuch as the plants in 1441 and 1534 of the same table were looking better after 28 days without changing than those in 1495 which had been changed twice a week. After about 10 days, the plants grown in a medium containing a dead culture of organisms exhibited very serious effects. Mockeridge, on the other hand, found that dead cultures of certain species of organisms stimulated the growth of Lemna. It might be pointed out again that 0.1 cc. of the sterile bouillon, the bacterial medium, had no effect on the plant, when used without dead organisms.

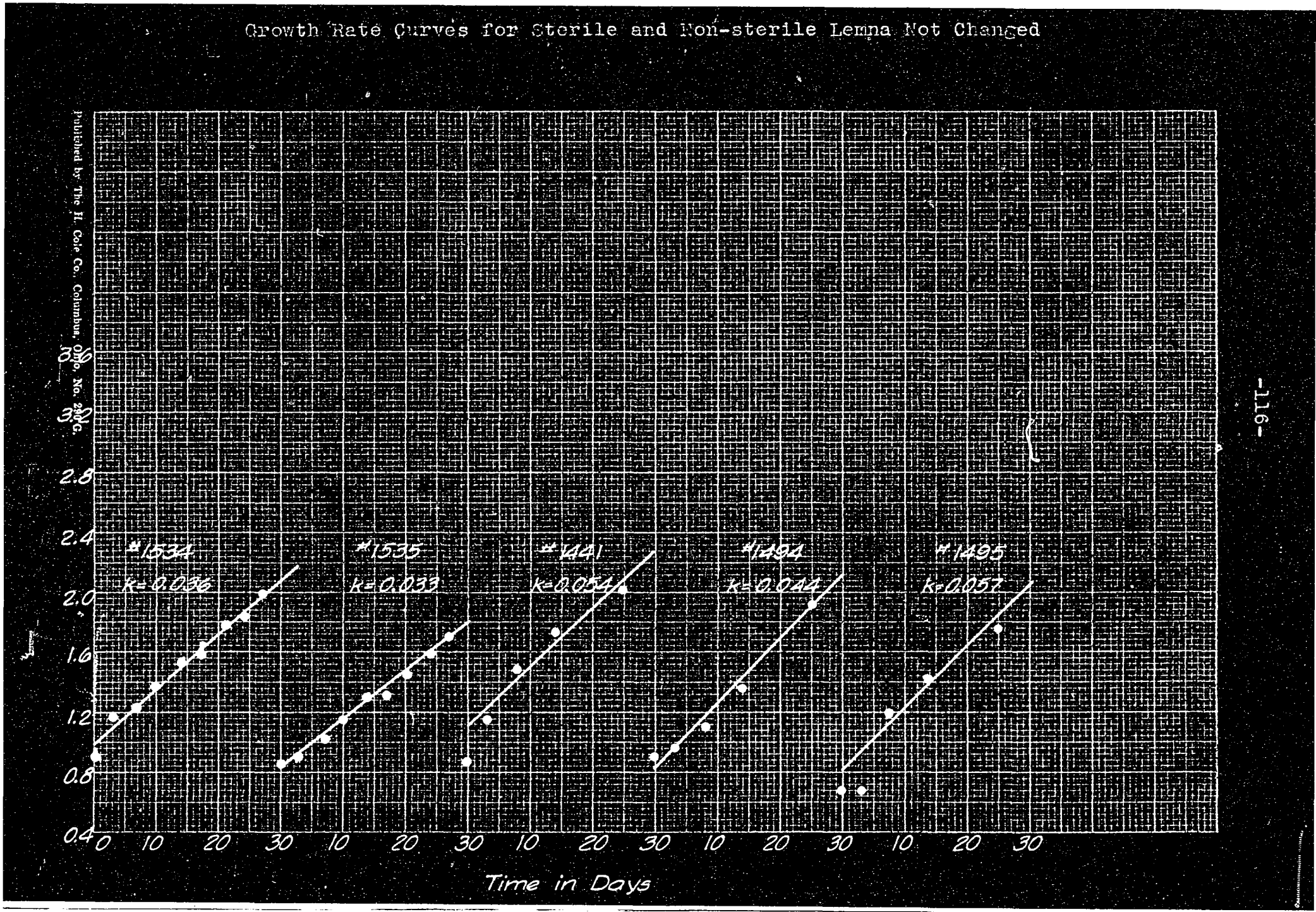

Growth Rate Curves for Sterile and Non-sterile Lemna Not Changed
Published by The H. Cole Co. Columbus, Ohio. No. 280 G.
Log. N
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
#1534
k=0.036
#1535
k=0.033
#1441
k=0.054
#1494
k=0.044
#1495
k=0.057
0 10 20 30 10 20 30 10 20 30 10 20 30 10 20 30
Time in Days

TABLE XIII. STERILE AND NON-STERILE LEMNA NOT CHANGED.

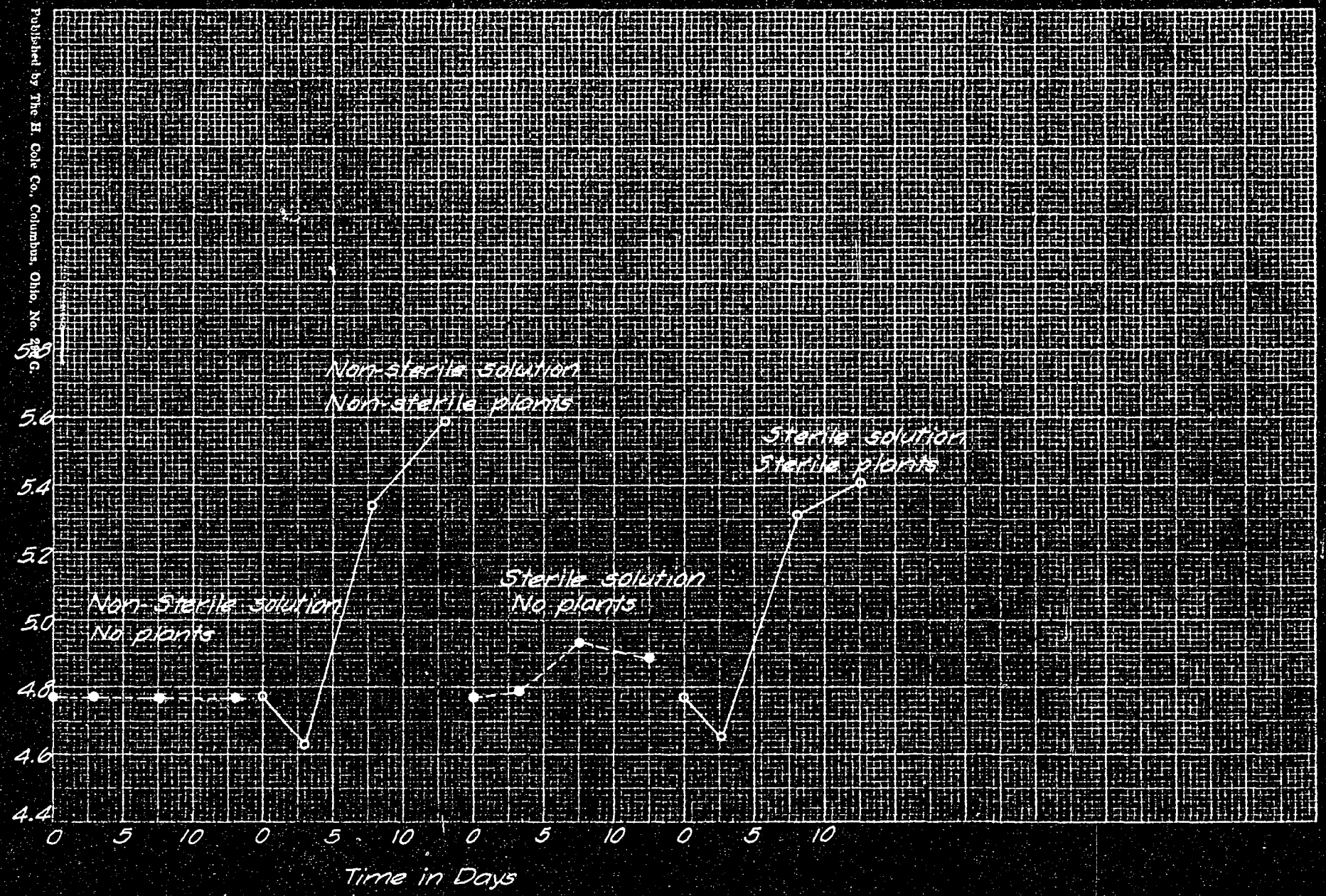
Number	Treatment	Plants from stock	k	pH at beginning	pH at end	Appearance at end	Sterility at end	Duration of experiment in days
1441	Modified Soln.1 Sterilized--not changed.	Sterile	0.054	4.80	5.35	a-4	s	54
1494	Modified Soln.1 " --not changed.	Non-sterile	0.044	4.80	5.58	a-1	n.s	24
1534	Modified Soln.1 " --not changed.	Sterile	0.036	4.80	5.30	a-4	n.s	28
1535	Modified Soln.1 " --not changed.	Non-sterile	0.033	4.80	5.40	a-1	n.s	28
1495	Modified Soln.1 + 0.1 cc.1476 Sterilized, changed twice a week.	Sterile	0.057	4.80	5.21	a-5	s	28

a = appearance of plants in sterile check in Table XII.
1476 is found in Table X.

PH OF STERILE AND NON-STERILE SOLUTIONS WITH AND WITHOUT LEMNA.

The following graph indicates that a non-sterile solution does not change in pH upon standing, while the pH of a sterile solution does not remain constant as one would anticipate, its change over a period of 13 days is relatively small. As is illustrated in the accompanying graph, both curves for sterile and non-sterile solutions with plants indicate that at first the pH decreases to a minimum after which there is a rapid increase. In 13 days there is produced a higher pH in the non-sterile solution than the sterile.

Curves Showing Change of pH of Sterile and Non-sterile Solutions With and Without Plants With Lime



Published by The H. Cole Co. Columbus, Ohio No. 288 G.

THE EFFECTS OF VARIOUS ORGANIC EXTRACTS ON YEAST GROWTH[*]

The object of this experiment was to determine the presence of a yeast stimulant in various extracts, and to study the relation of micro-organisms present on the plant to the production of a yeast stimulant.

The basal solution used in these experiments for yeast growth was medium C (23,24) whose composition is as follows:

NH_4Cl----------0.188 grs.
KH_2PO_4----------0.100 "
Cane sugar----------10.000 "
Distilled water------enough to make
100 cc. of solution.

In order to permit the addition of the organic extract and of the yeast suspension to Medium C without dilution of the medium, the above substances were dissolved in only 90 cc. of distilled water rather than being dissolved in 100 cc. of solution. After the yeast and extract were added, the solution was then diluted to 100 cc. Each 100 cc. of solution used for yeast growth, then, finally had the following composition:

100 cc. of Medium C

1 Organic extract (p.p.m.)
2 Yeast cells (count of one)
3 NH_4Cl----------0.188 grs.
KH_2PO_4----------0.100 "
Cane sugar--------10.000 "

---

[*]Through the courtesy of the Department of Biochemistry, and by the cooperation of Mr. H. Schopmeyer, it was possible for us to obtain these results.

The above solution, Medium C, containing varying quantities of various organic extracts were inoculated with yeast with a count of one (43). Each cubic centimeter of the 100 cc. of the solution after inoculation, then contained 250,000 yeast cells (43). The solutions were incubated at 30°C. for 48 hours and counted (43). As represented by the tables and graphs, each cubic centimeter of the medium then, contained the count x11/10x250,000 yeast cells. The yeast used for inoculating was Saccharomyces cereviseae, grown by Fulmer for several years.

The following tables and graphs show the results of these experiments.

Effects of Bios. I on Yeast Growth

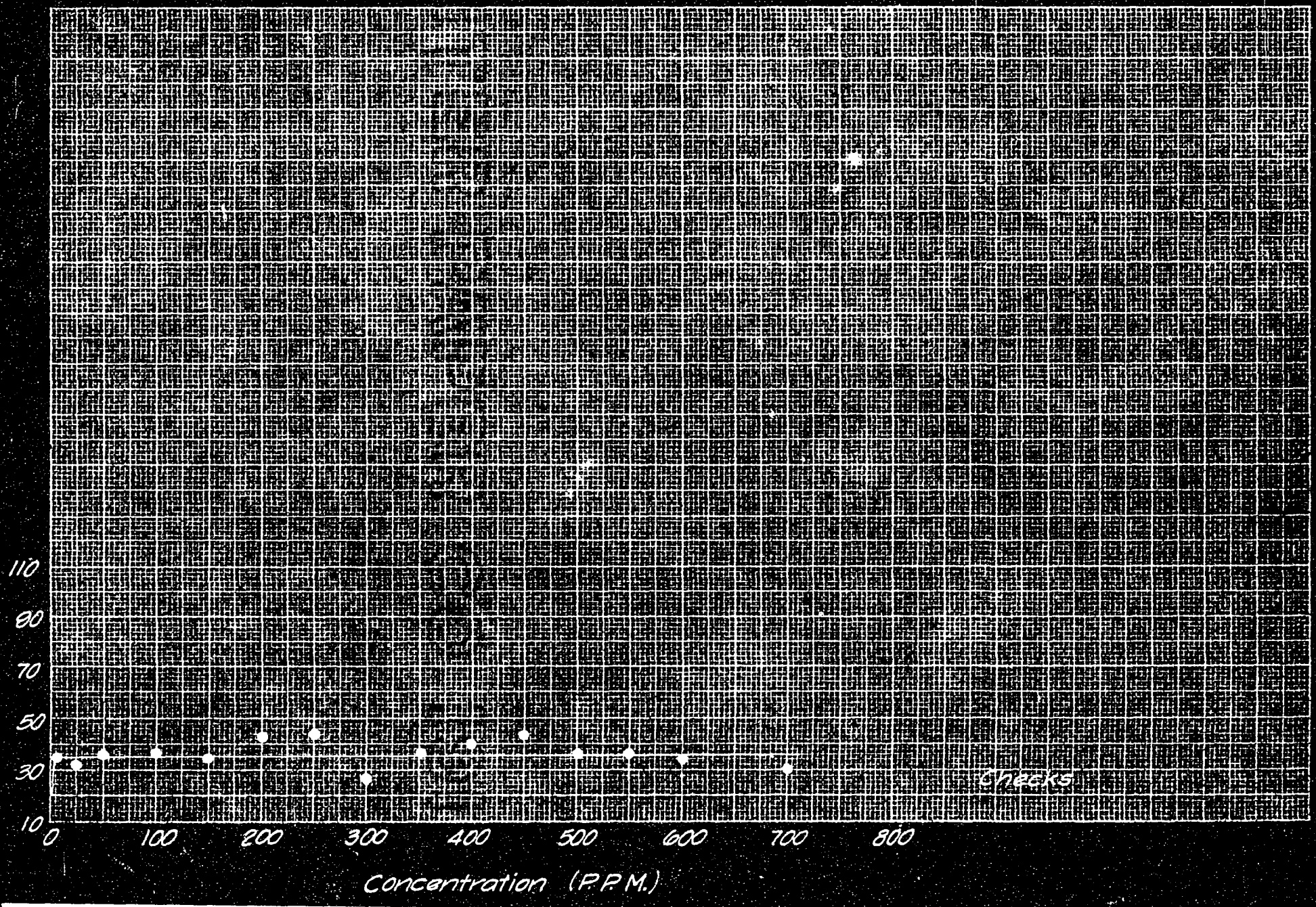


TABLE XIV. EFFECTS OF BIOS I ON YEAST GROWTH.

Number	Bios I added p.p.m.	Yeast added	Medium: C used	Count after 48 hours
1	5	Count of one	100 cc	34 x 6/10 x 250,000 per cc.
2	25	" " "	100 "	31 x 6/10 x 250,000 " "
3	50	" " "	100 "	35 x 6/10 x 250,000 " "
4	100	" " "	100 "	36 x 6/10 x 250,000 " "
5	150	" " "	100 "	33 x 6/10 x 250,000 " "
6	200	" " "	100 "	43 x 6/10 x 250,000 " "
7	250	" " "	100 "	43 x 6/10 x 250,000 " "
8	300	" " "	100 "	26 x 6/10 x 250,000 " "
9	350	" " "	100 "	36 x 6/10 x 250,000 " "
10	400	" " "	100 "	40 x 6/10 x 250,000 " "
11	450	" " "	100 "	43 x 6/10 x 250,000 " "
12	500	" " "	100 "	37 x 6/10 x 250,000 " "
13	550	" " "	100 "	38 x 6/10 x 250,000 " "
14	600	" " "	100 "	35 x 6/10 x 250,000 " "
15	700	" " "	100 "	31 x 6/10 x 250,000 " "
x	Check	" " "	100 "	19 x 6/10 x 250,000 " "
x	"	" " "	100 "	20 x 6/10 x 250,000 " "

Bios I was obtained as follows:

1600 grams of alfalfa were steeped with distilled water at 70°C. for 4 hours, filtered, and again steeped at 70°C. The filtrates were then concentrated in vacuo at 40°C. and 30 m.m. pressure and were made 40% by volume with ethyl alcohol. The precipitate thus formed was washed repeatedly with 40% alcohol, and air dried. This Bios I is cream colored and only slightly soluble in water.

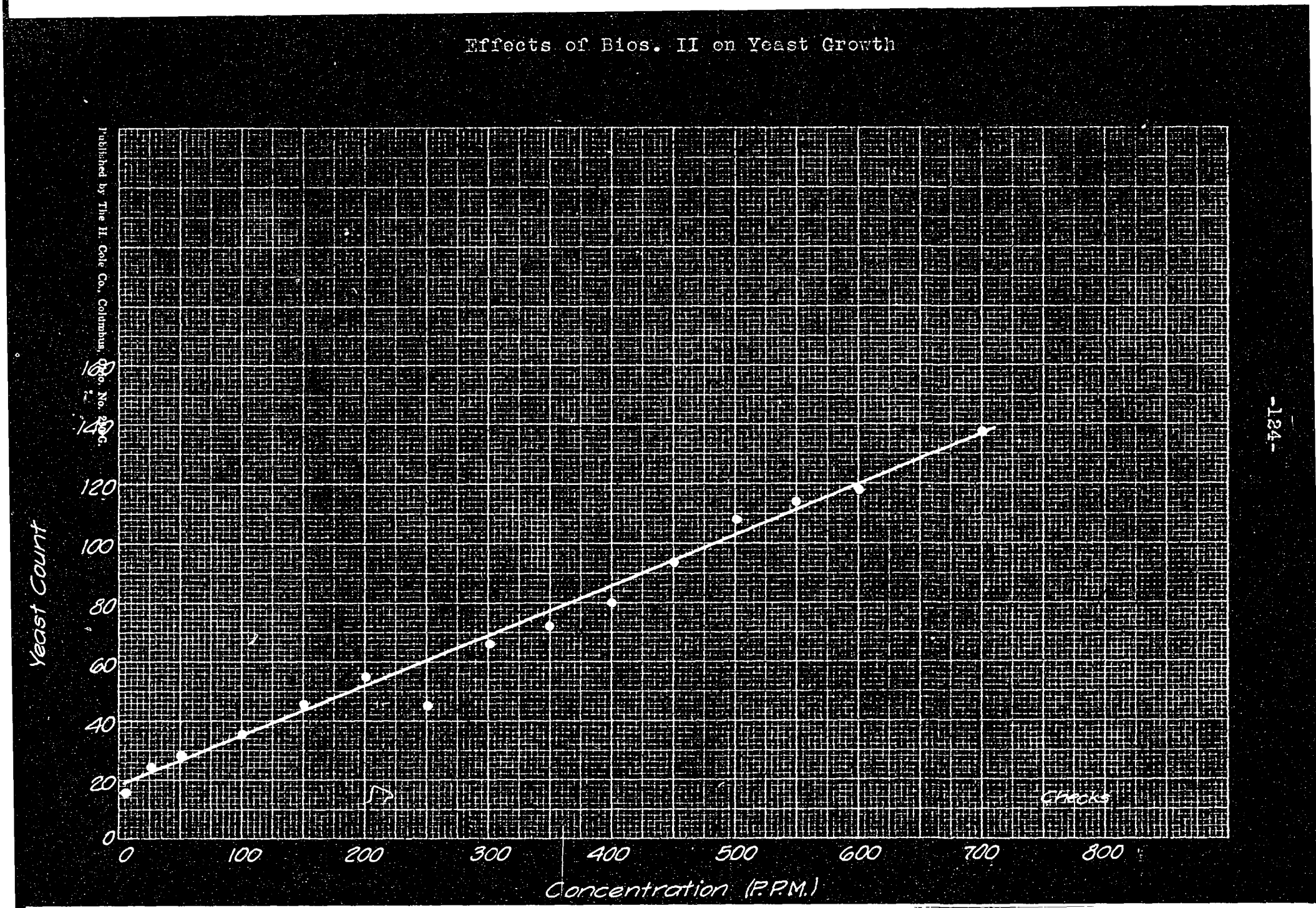

Effects of Bios. II on Yeast Growth
Yeast Count
0
20
40
60
80
100
120
140
160
0
100
200
300
400
500
600
700
800
Concentration (P.P.M.)
Glucose
Published by The H. Cole Co. Columbus, O. No. 250 G.

TABLE XV. EFFECTS OF BIOS II ON YEAST GROWTH.

Number	Bios II added p.p.m.	Yeast added	Medium: C used	Count after 48 hours
1	5	Count of one	100 cc	18 x 11/10 x 250,000 per cc.
2	25	" " "	100 cc	24 x 11/10 x 250,000 " "
3	50	" " "	100 cc	29 x 11/10 x 250,000 " "
4	100	" " "	100 cc	35 x 11/10 x 250,000 " "
5	150	" " "	100 cc	47 x 11/10 x 250,000 " "
6	200	" " "	100 cc	55 x 11/10 x 250,000 " "
7	250	" " "	100 cc	46 x 11/10 x 250,000 " "
8	300	" " "	100 cc	66 x 11/10 x 250,000 " "
9	350	" " "	100 cc	71 x 11/10 x 250,000 " "
10	400	" " "	100 cc	80 x 11/10 x 250,000 " "
11	450	" " "	100 cc	94 x 11/10 x 250,000 " "
12	500	" " "	100 cc	107 x 11/10 x 250,000 " "
13	550	" " "	100 cc	115 x 11/10 x 250,000 " "
14	600	" " "	100 cc	117 x 11/10 x 250,000 " "
15	700	" " "	100 cc	137 x 11/10 x 250,000 " "
x	Check	" " "	100 cc	10 x 11/10 x 250,000 " "
x	"	" " "	100 cc	12 x 11/10 x 250,000 " "

Bios II was obtained as follows:

The filtrate from Bios I was made 70% by volume with ethyl alcohol. The brown precipitate thus formed was repeatedly washed with 70% alcohol, and dried at 65°C. in vacuo. This fraction is very soluble in water.

Effects of Bios. III on Yeast Growth

Published by The H. Reble Co., Columbus, Ohio. No. 296-G.

TABLE XVI. EFFECTS OF BIOS III ON YEAST GROWTH

Number	Bios III added p.p.m.	Yeast added	Medium C used	Count after 48 hours
1	5	Count of one	100 cc	24 x 11/10 x 250,000 per cc.
2	25	" " "	100 "	30 x 11/10 x 250,000 " "
3	50	" " "	100 "	55 x 11/10 x 250,000 " "
4	100	" " "	100 "	84 x 11/10 x 250,000 " "
5	150	" " "	100 "	102x 11/10 x 250,000 " "
6	200	" " "	100 "	146x 11/10 x 250,000 " "
7	250	" " "	100 "	158x 11/10 x 250,000 " "
8	300	" " "	100 "	152x 11/10 x 250,000 " "
9	350	" " "	100 "	151x 11/10 x 250,000 " "
10	400	" " "	100 "	161x 11/10 x 250,000 " "
11	450	" " "	100 "	168x 11/10 x 250,000 " "
12	500	" " "	100 "	162x 11/10 x 250,000 " "
13	550	" " "	100 "	179x 11/10 x 250,000 " "
14	600	" " "	100 "	173x 11/10 x 250,000 " "
15	700	" " "	100 "	163x 11/10 x 250,000 " "
x	Check	" " "	100 "	11 x 11/10 x 250,000 " "
x	"	" " "	100 "	12 x 11/10 x 250,000 " "

Bios III was obtained as follows:

The filtrate from Bios II was made 95% by volume with absolute alcohol. The brown precipitate formed was washed repeatedly with 95% alcohol, and dried at 65°C. in vacuo. This fraction is very soluble in hot water and it is very hydroscopic.

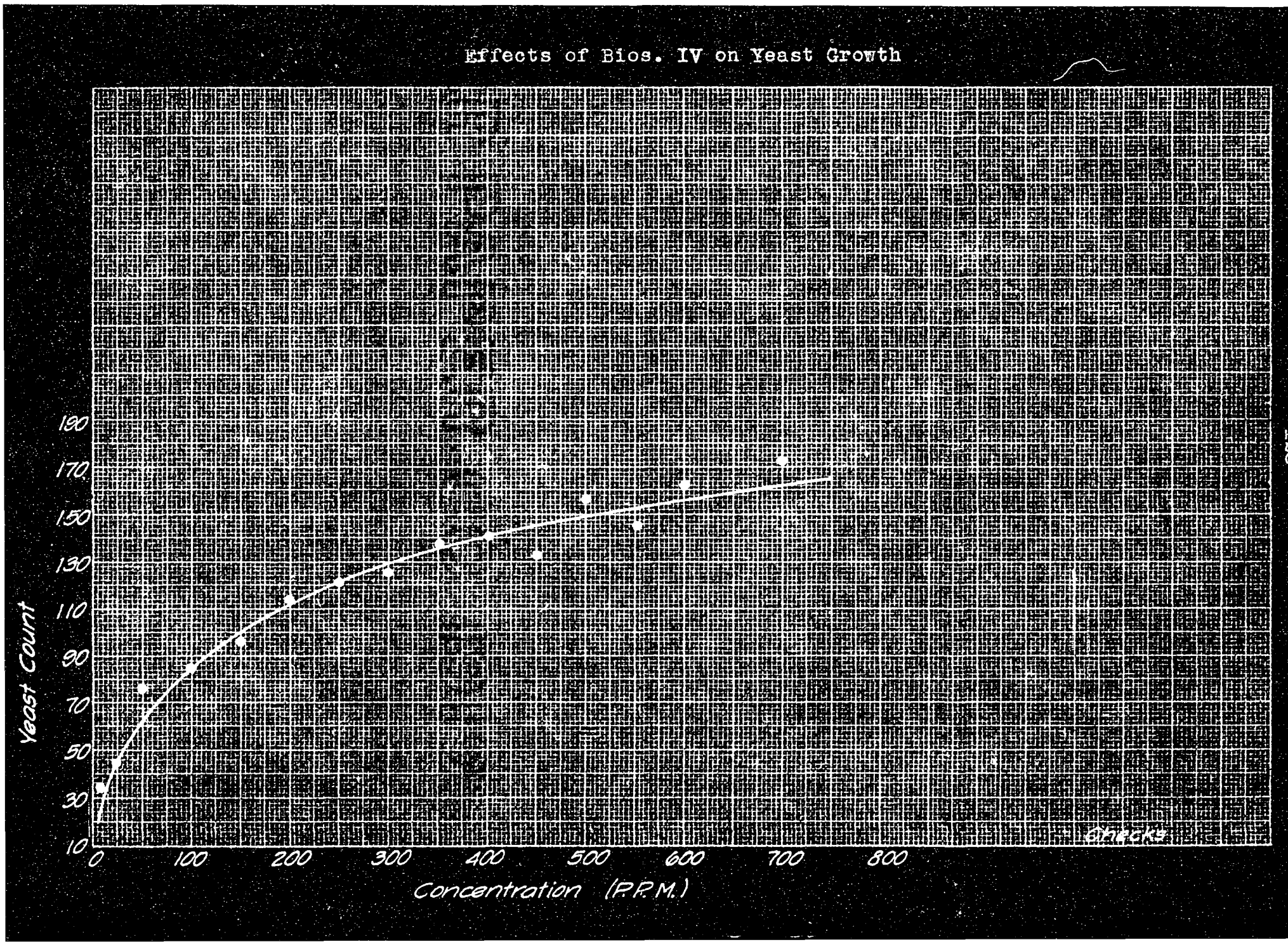

Effects of Bios. IV on Yeast Growth
Yeast Count
190
170
150
130
110
90
70
50
30
10
0
100
200
300
400
500
600
700
800
Concentration (P.P.M.)

TABLE XVII. EFFECTS OF BIOS IV ON YEAST GROWTH.

Number	Bios IV added p.p.m.	Yeast added	Medium C used	Count after 48 hours
1	5	Count of one	100 cc	34 x 11/10 x 250,000 per cc.
2	25	" " "	100 "	45 x 11/10 x 250,000 " "
3	50	" " "	100 "	76 x 11/10 x 250,000 " "
4	100	" " "	100 "	85 x 11/10 x 250,000 " "
5	150	" " "	100 "	95 x 11/10 x 250,000 " "
6	200	" " "	100 "	114 x 11/10 x 250,000 " "
7	250	" " "	100 "	120 x 11/10 x 250,000 " "
8	300	" " "	100 "	124 x 11/10 x 250,000 " "
9	350	" " "	100 "	138 x 11/10 x 250,000 " "
10	400	" " "	100 "	140 x 11/10 x 250,000 " "
11	450	" " "	100 "	133 x 11/10 x 250,000 " "
12	500	" " "	100 "	156 x 11/10 x 250,000 " "
13	550	" " "	100 "	146 x 11/10 x 250,000 " "
14	600	" " "	100 "	162 x 11/10 x 250,000 " "
15	700	" " "	100 "	172 x 11/10 x 250,000 " "
x	Check	" " "	100 "	11 x 11/10 x 250,000 " "
x	"	" " "	100 "	12 x 11/10 x 250,000 " "

Bios IV was obtained as follows:

The filtrate from Bios III was evaporated in vacuo at 65°C. and 30 m.m. This fraction is very soluble in water and is very hydroscopic.

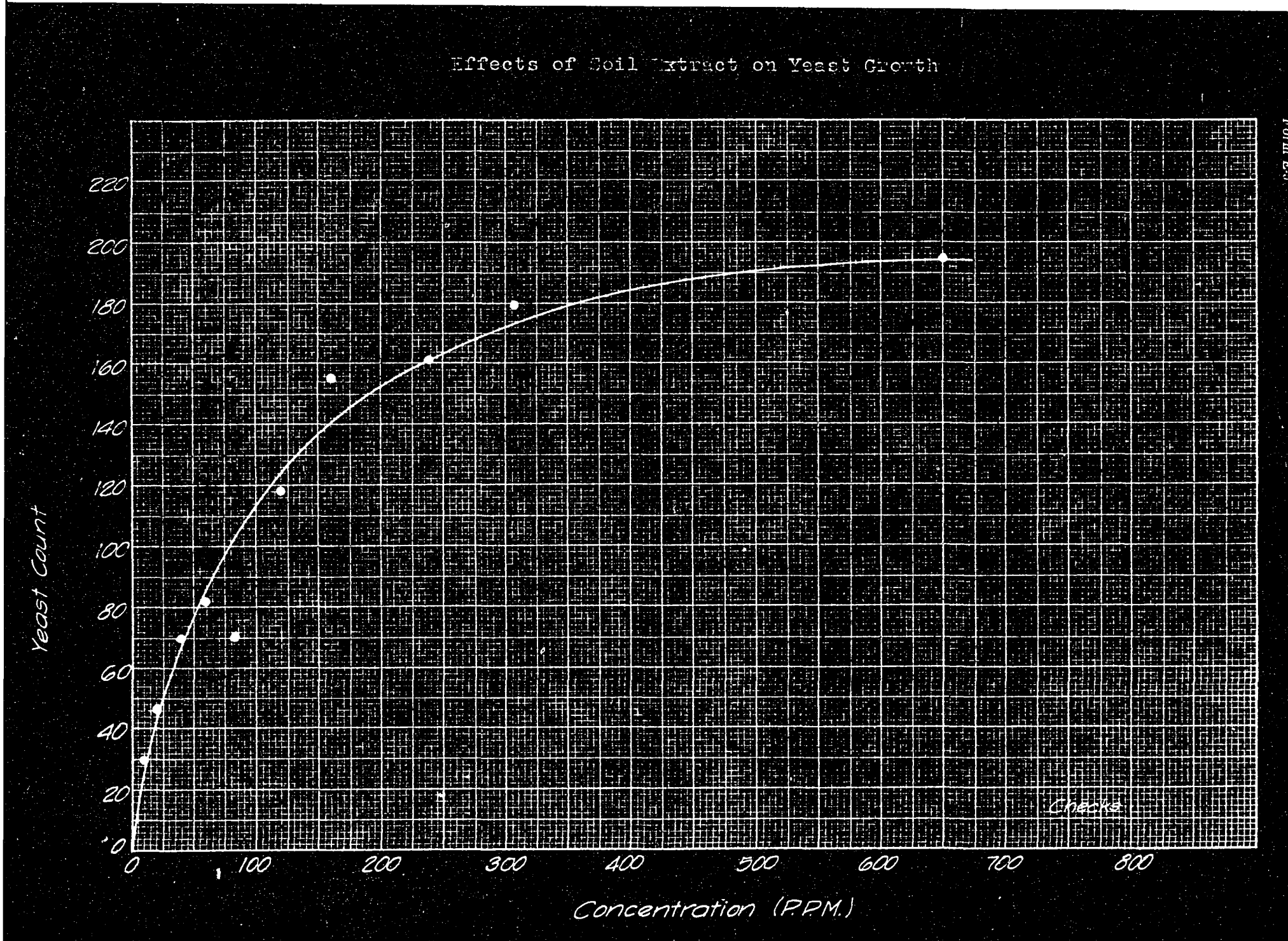

Effects of Soil Extract on Yeast Growth
Yeast Count
0
20
40
60
80
100
120
140
160
180
200
220
0
100
200
300
400
500
600
700
800
Concentration (P.P.M.)
Checks

TABLE XVIII. EFFECTS OF SOIL EXTRACT ON YEAST GROWTH.

Number	Soil extract added p.p.m.	Yeast added	Medium C used	Count after 48 hours
1	10	Count of one	100cc	31 x 11/10 x 250,000 per cc.
2	20	" " "	100 "	48 x 11/10 x 250,000 " "
3	40	" " "	100 "	72 x 11/10 x 250,000 " "
4	60	" " "	100 "	83 x 11/10 x 250,000 " "
5	80	" " "	100 "	70 x 11/10 x 250,000 " "
6	120	" " "	100 "	118 x 11/10 x 250,000 " "
7	160	" " "	100 "	156 x 11/10 x 250,000 " "
8	240	" " "	100 "	161 x 11/10 x 250,000 " "
9	320	" " "	100 "	183 x 11/10 x 250,000 " "
10	640	" " "	100 "	194 x 11/10 x 250,000 " "
x	Check	" " "	100 "	11 x 11/10 x 250,000 " "
x	"	" " "	100 "	11 x 11/10 x 250,000 " "

The soil extract was obtained as previously described in this thesis.

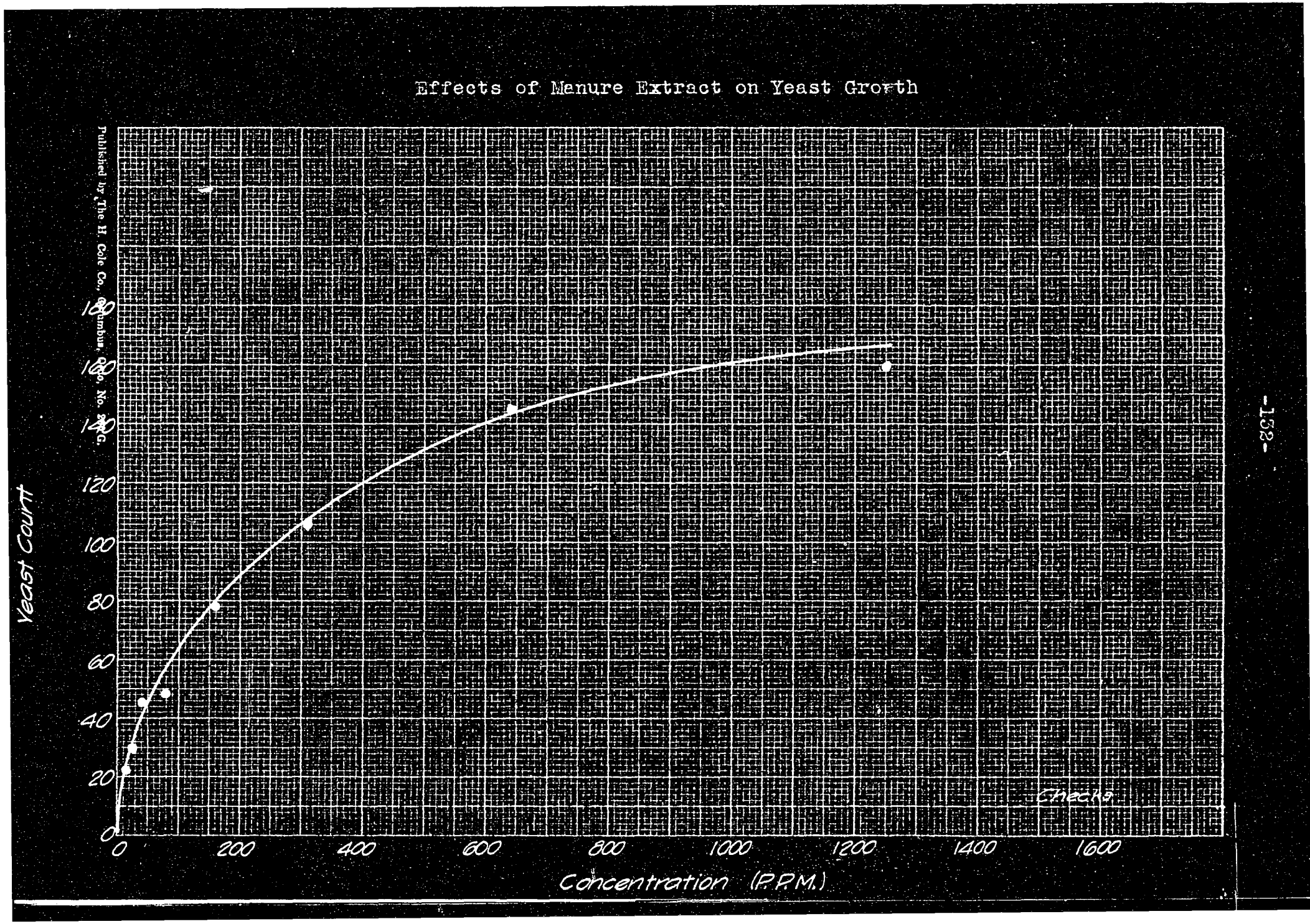

Effects of Manure Extract on Yeast Growth
Published by The H. Cole Co., Columbus, Ohio. No. 340 G.
Yeast Count
0
20
40
60
80
100
120
140
160
180
0
200
400
600
800
1000
1200
1400
1600
Concentration (P.P.M.)
Checks

TABLE XIX: EFFECT OF MANURE EXTRACT ON YEAST GROWTH.

Number	Manure extract added p.p.m.	Yeast added	Medium: C used:	Count after 48 hours
1	10	Count of one	100 cc:	23 x 11/10 x 250,000 per cc.
2	20	" " "	100 cc:	29 x 11/10 x 250,000 " "
3	40	" " "	100 cc:	46 x 11/10 x 250,000 " "
4	80	" " "	100 cc:	48 x 11/10 x 250,000 " "
5	160	" " "	100 cc:	78 x 11/10 x 250,000 " "
6	320	" " "	100 cc:	107 x 11/10 x 250,000 " "
7	640	" " "	100 cc:	145 x 11/10 x 250,000 " "
8	1280	" " "	100 cc:	160 x 11/10 x 250,000 " "
x	Check	" " "	100 cc:	11 x 11/10 x 250,000 " "
x	"	" " "	100 cc:	11 x 11/10 x 250,000 " "

The manure extract was obtained as previously described in this thesis.

Effects of Non-sterile Lemna Extract on Yeast Growth

Form E-3

TABLE XX. EFFECT OF NON-STERILE LEMNA EXTRACT ON YEAST GROWTH.

Number	Non-sterile Lemna extract added p.p.m.	Yeast added	Medium C used	Count after 48 hours
1	10	Count of one	100 cc	23 x 11/10 x 250,000 per cc.
2	20	" " "	100 "	29 x 11/10 x 250,000 " "
3	40	" " "	100 "	44 x 11/10 x 250,000 " "
4	60	" " "	100 "	55 x 11/10 x 250,000 " "
5	80	" " "	100 "	69 x 11/10 x 250,000 " "
6	100	" " "	100 "	83 x 11/10 x 250,000 " "
7	120	" " "	100 "	88 x 11/10 x 250,000 " "
8	140	" " "	100 "	98 x 11/10 x 250,000 " "
9	160	" " "	100 "	106 x 11/10 x 250,000 " "
10	240	" " "	100 "	118 x 11/10 x 250,000 " "
11	320	" " "	100 "	124 x 11/10 x 250,000 " "
12	480	" " "	100 "	127 x 11/10 x 250,000 " "
13	640	" " "	100 "	-- x 11/10 x 250,000 " "
x	Check	" " "	100 "	11 x 11/10 x 250,000 " "
x	"	" " "	100 "	11 x 11/10 x 250,000 " "

The non-sterile Lemna extract was obtained by treating about a gram of the dried plants with distilled water at 70°C. for two hours, filtering and repeating the operations with the residue. These two filtrates were collected, made up to volume, and used in the above experiment.

Effects of Sterile Lemna Extract on Yeast Growth

TABLE XXI. EFFECTS OF STERILE LEMNA EXTRACT ON YEAST GROWTH.

Number	Sterile Lemna extract added p.p.m.	Yeast added	Medium C used	Count after 48 hours
1	3	Count of one	100 cc	20 x 11/10 x 250,000 per cc.
2	7	" " "	100 "	29 x 11/10 x 250,000 " "
3	10	" " "	100 "	30 x 11/10 x 250,000 " "
4	13	" " "	100 "	41 x 11/10 x 250,000 " "
5	20	" " "	100 "	49 x 11/10 x 250,000 " "
6	27	" " "	100 "	50 x 11/10 x 250,000 " "
7	33	" " "	100 "	55 x 11/10 x 250,000 " "
8	40	" " "	100 "	62 x 11/10 x 250,000 " "
9	47	" " "	100 "	70 x 11/10 x 250,000 " "
10	53	" " "	100 "	86 x 11/10 x 250,000 " "
11	80	" " "	100 "	90 x 11/10 x 250,000 " "
12	105	" " "	100 "	82 x 11/10 x 250,000 " "
13	160	" " "	100 "	81 x 11/10 x 250,000 " "
x		" " "	100 "	11 x 11/10 x 250,000 " "
x		" " "	100 "	11 x 11/10 x 250,000 " "

The sterile Lemna extract was obtained by treating about one-half gram of the dried sterile plants in the same manner as the non-sterile Lemna was treated for an aqueous extract. This extract was used in the above experiment.

The results show that Bios I is not as good a yeast stimulant as the other three fractions. Bios III and IV are better than II. These results roughly check those of Fulmer, Duecker and Nelson (22). The action of these fractions on the sterile Lemna is almost the reverse of that action shown with yeast. It is noticed that the activity of the soil extract on yeast is slightly better than that of the manure, while neither extract shows a marked stimulating power over fractions III and IV. Though the non-sterile plant extract does not have the marked effects on yeast growth as do some of the other extracts, it does, however, increase the growth rate. The sterile Lemna extract shows a decreased power of stimulation as compared to the non-sterile Lemna, however, the sterile plant extract does stimulate yeast growth. It would appear, therefore, that the sterile Lemna plant can manufacture this stimulant, and that micro-organisms are not necessary for its production.

CONCLUSIONS

It has been recognized for some time that some organic materials will stimulate the growth of green plants, and a few investigators believed that minute quantities of organic matter are essential. The nature of the stimulation by organic substances was not thoroughly understood, yet their presence increased the growth rate of the plant. Only recently, when the Lemna plant has been made to reproduce and grow healthily in a solution of purified inorganic salts was it apparent that minute quantities of organic matter are not essential for the satisfactory growth of the green plant. This left unsettled, however, the question of the effects of organic substances on the green plant. In order to study this question more thoroughly, we sterilized Lemna, and studied the effects of organic matter on the sterile as well as the non-sterile plant.

When both sterile and non-sterile plants were grown separately in a sterile inorganic medium of the same composition, we unexpectedly found that the sterile plant grew better than the non-sterile. It is quite evident, then, from the results of this work that micro-organisms are not only non-essential for the growth of Lemna, but that they are harmful. The next unexpected result was that the sterile organic matter we used did not stimulate the growth of sterile Lemna over that of the sterile check grown in a sterile in-

organic medium, but in some cases it actually depressed the reproduction rate of the Lemna plant. The opposite result was true of the non-sterile plant, in that nearly all organic substances used increased its rate of reproduction. Therefore, there are indications that, while the sterile plant grows faster than the non-sterile, when both are grown separately in a sterile inorganic medium, organic matter will not stimulate the growth of sterile Lemna unless micro-organisms are present; also in some cases the rate of reproduction is greater than either the sterile or non-sterile checks. Upon both the sterile and the non-sterile plant, regardless of whether there was stimulation or depression of the growth rate, nearly every organic substance exhibited a point of optimum concentration. The beneficial effects on the plant of adding micro-organisms to sterile organic matter containing sterile Lemna may be due to the changing of the activity of the organisms from the less available organic matter of the plant to the more available form which was added, or to the action of the organisms on the added organic matter, making it available to the plants, or to both of these factors. Inasmuch as sterile organic matter was either harmful or ineffective to the sterile plant, one would expect a dead culture of organisms obtained from the non-sterile plant to fall into one of these classes. As a matter of fact, such a culture was decidedly toxic.

It is evident that micro-organisms are not essential to the plant in its manufacture of a yeast stimulant.

The optimum pH for Lemna, while not the same for all solutions, is very important if the plant is to be grown successfully.

SUMMARY

1. An inorganic solution is described in which Lemna major has reproduced and has grown healthily through several hundred generations, without the presence of organic matter.

2. Four inorganic solutions are described in which the green plant has grown satisfactorily.

3. The optimum pH for each of the four inorganic solutions was determined.

4. The methods and technic used in all nutrient solution work are described.

5. The attempts to sterilize Lemna and its subsequent sterilization and growth are described. The sterile plant was demonstrated to have grown in a sterile solution containing purified inorganic salts for several months, and to have continued in a healthy state.

6. Aqueous extracts of yeast, barley, alfalfa, soil and manure at 80 p.p.m. stimulated the growth of Lemna, and depressed its growth at 40 p.p.m., but an aqueous extract of carrots depressed the growth rate of the plant at both 80 and 40 p.p.m.

7. An optimum concentration for the stimulating action of manure extract was found to be approximately 80 p.p.m; of soil extract, 20 and 65 p.p.m., of alfalfa extract, 180 p.p.m., of urea, 60 p.p.m., and of asparagin, 4 p.p.m. No optimum was found for soil suspension, while for manure suspension, there was no stimulation between 3 - 200 p.p.m.

8. Soil extract stimulated growth in all concentrations from 1 - 200 p.p.m., manure extract showed an optimum stimulation at 2 p.p.m., a minimum depression at 4 p.p.m., and a second optimum stimulation at 80 p.p.m., and alfalfa extract showed a minimum depression at 8 p.p.m.

9. The sterile plant was not stimulated by any sterile organic substance used. Manure and soil extracts, Bios I and III showed a point of optimum concentration in the growth of sterile Lemna, but in no case was the optimum greater than that of the sterile inorganic check.

10. When both sterile and non-sterile plants were grown in the sterile inorganic medium of the same composition, the sterile plant grew faster and appeared healthier than the non-sterile.

11. When a culture of organisms which had been isolated from the non-sterile plant were added to sterile Lemna in sterile modified solution 1 containing organic matter, stimulation of growth rate over that of sterile check was at once noticed. This was the case for all organic extracts used, whether the non-sterile plant or the organic extract were used as a source of inoculation.

12. When the sterile plants growing in the sterile inorganic solution are inoculated with organisms from the non-sterile plant, the growth rate is decreased until it reaches that for non-sterile Lemna.

13. A dead culture of organisms from the non-sterile plant

is toxic to the sterile Lemna growing in the sterile inorganic medium.

14. Two hundred parts per million of urea in modified solution 1 was harmful to Lemna both under sterile and non-sterile conditions. The same concentration of sterile acetamide and creatinine had no effect on the plant, but these solutions under non-sterile conditions were harmful.

15. Of the ten pure cultures of living bacteria used in inoculating the sterile modified solution 1 containing manure extract, none decreased the rate of reproduction of sterile Lemna. In fact, all organisms except Bacterium coli and Number 11 increased the growth rate of the sterile green plant.

16. A water suspension of sterile Carrington loam soil slightly depresses the growth rate of sterile Lemna, but when a similar suspension was inoculated with a small particle of the non-sterile soil, the rate was greatly increased over that of the sterile inorganic check.

17. The sterile and the non-sterile plants can be grown continuously for about ten days in separate portions of solution 1 without serious effects, but the non-sterile plant suffers less from this treatment.

18. The pH of the non-sterile medium containing no plants did not change over a period of time; the sterile medium, on the other hand, increased slightly. The non-sterile, and sterile solutions containing plants first decreased to a minimum, then later increased rapidly in pH. The non-sterile so-

lution with plants developed a higher pH than did the sterile solution with plants.

19. All organic extracts used stimulated yeast growth. Bios I was a poor stimulant.

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