1931

Biochemical relations between iron and manganese, and organic matter in the growth of the green plant

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NOTE TO USERS

BIOCHEMICAL RELATIONS BETWEEN IRON AND MANGANESE, AND ORGANIC MATTER IN THE GROWTH OF THE GREEN PLANT

By

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A Thesis Submitted to the Graduate Faculty

for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject Soil Chemistry

Approved:

Signature was redacted for privacy.

In Charge of Major Work

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1931

ACKNOWLEDGEMENTS

The writer wishes to express his appreciation of the cooperation of Dr. Norman A. Clark, not only in directing this work but in checking some of the more difficult points in his own laboratory.

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THE ROLE OF MANGANESE IN THE NUTRITION OF LEMNA

This part of the thesis was published jointly with Dr. Norman A Clark in Plant Physiology 2, 241-248, 1930. A reprint is inserted here for convenience. Further studies on manganese which have been carried out in connection with the work on iron and organic matter, are discussed under those particular sections. A summary of the findings on manganese is given at the end of this thesis.

THE ROLE OF MANGANESE IN THE NUTRITION OF LEMNA

NORMAN ARNWELL CRAIG AND CHARLES A. REA

Reprinted from PLANT PHYSIOLOGY, 21: 241-248, 1946.

Reprinted from PLANT PHYSIOLOGY, 2: 241–248, 1930.

THE RÔLE OF MANGANESE IN THE NUTRITION OF LEMNA[1]

NORMAN ASHWELL CLARK AND CLAUDE L. FLY

(WITH THREE FIGURES)

The place of manganese in the nutrition of the green plant has been investigated in detail for some years, and numerous claims have been advanced with regard to its function, but there does not seem to be any considerable consensus of opinion as to its effect on the composition of the tissues or on the reproduction of the plant. There seems to be an equal uncertainty whether manganese should be classed as one of those elements which have been claimed as essential for growth (although in small quantities), or if it should be put with those which stimulate but are not absolutely necessary for the existence of the plant.

In 1920 OLARU published a thesis on the place of manganese in agriculture (**15**) and reviewed the work done throughout the world up to that time. In almost all cases manganese had proved favorable to the development of plants in soils, although large quantities were decidedly toxic. In liquid cultures the addition of suitable quantities of manganese stimulated both germination and development.

OLARU showed that this energetic action of manganese had been explained by the many different investigators, in almost as many different ways, including among others: catalysis of chemical action in the cell (BERTRAND); oxidation of toxic substances in the leaves (LOEW); production of chlorophyll (VAN DORN); rôle in photosynthesis (STOKLASA); stimulation of assimilation of elements (ROUSSET); chemical action on soil salts (MENOZZI); increased solubility of calcium salts and silicates (BERNARDINI); increased oxidation (SCHREINER); oxidation and stimulation of bacteria (SKINNER); permeability of protoplasm of cell to salts of calcium and magnesium (KELLY); ratio of iron to manganese (PUGLIESE).

By 1925 the amount of information had increased but without altering to any large extent the conclusions of OLARU. Influenced by the discussion on the essential nature of the vitamins in animal life, investigators gave more attention to the place of manganese as a possible essential element in plant life, in contrast to its stimulating action. BRENCHLEY (**5**) in her monograph on Inorganic Plant Poisons and Stimulants in 1927 sums up that manganese has a stimulating action on growth in small quantities, but large amounts are toxic to plants. The precise way in which this stimulation is accomplished is not known, and the "essential" nature of the element is as yet uncertain.

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

Several workers have recently reported experiments on plants for which manganese seemed an essential. HAAS and REED (**12**) found that with young orange trees they could not get good growth in culture solutions commonly considered "complete," when the young trees were grown for long periods in these solutions. Manganese was among the elements which it was necessary to add in traces to remove this condition. BISHOP (**4**) in Australia noted the presence of manganese in 25 species of Eucalypts. In these and in wheat, barley, maize, vegetables and fruits, the manganese was concentrated in the parts of the plants where there was the greatest chemical change. In his sand cultures he found approximately 5 parts per million was the optimum concentration in the nutrient solution supplied, but that neutralizing the acid solution with $CaCO_3$ enabled plants to grow in 50 ppm.

MCHARGUE (**14**) in 1927 reported the occurrence of a number of elements, including manganese, in Kentucky blue grass. From earlier experiments he had concluded that manganese is essential for the growth of plants, and is connected with chlorophyll or protein formation, or possibly with the stimulation of enzymes which split fats, sugars and starches, and render them available for young seedlings. He suggests here that it is the manganese which causes the deep blue color of the blue grass, and he proposes a theory connecting that element with vitamin production in plants. As most of the copper, manganese and zinc occurs in the germ of the seed, it may be connected with those vitamins which are found in the germ of the seed. Similarly the mineral elements supply the vitamin-forming material necessary for the growth of plants, and may become upon resynthesis the vitamin factors necessary for animal growth.

A number of these points can be studied very well in nutrient solutions. ASO (**3**) in 1902 used water cultures and stated that plants could develop normally in the absence of any trace of manganese, while WEIS (**17**) more recently states that manganese could not prevent chlorosis and that it did not influence the plants in solution. These conclusions are not in accord with those of HAAS, BISHOP or MCHARGUE.

The value of water plants for nutrient studies has been generally recognized, but the fact that they are specially adapted to the investigation of manganese has not been made use of to any extent. GÖSSL (**11**) showed that marsh and water plants generally gather up considerably more manganese than land plants; *Lemna trisulca,* for example, contained large amounts. In the experiments reported below, *Lemna major* (*Spirodela polyrhiza*) was used. As collected from ponds in central Iowa it showed a considerable amount of manganese in its composition.

Conditions under which the *Lemna* plants grow have been investigated in this laboratory for several years. In 1924 CLARK and ROLLER (**10**)

showed that the plants, if given a suitable medium of inorganic constituents, did not need the addition of organic matter in order to reproduce and to keep healthy, and that the "auximones" suggested by BOTTOMLEY were possibly of the nature of stimulants, but could not be classed as essentials for the growth of plants. Effects of intensity and duration of light on the reproduction were reported by CLARK in 1925 (**6**) and the influence of hydrogen ion concentration was investigated in 1926 (**7**). The medium developed in this way has been used by ASHBY and his coworkers (**1, 2**) in London with considerable success in the growth and reproduction of *Lemna minor*. A general review of some of the problems connected with vitamins and plant growth was given by CLARK in 1929 (**9**).

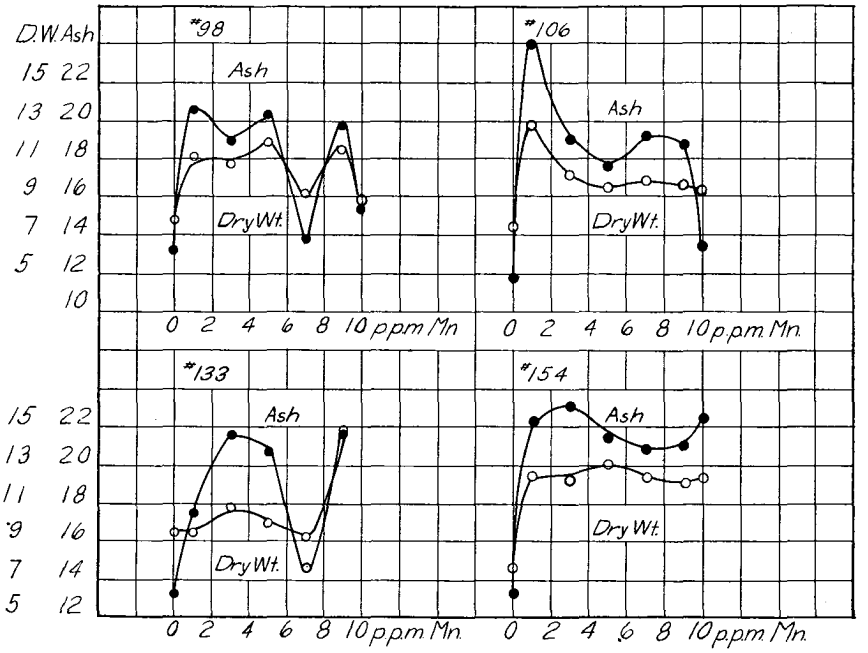

FIG. 1. Average dry weight and ash in plants with concentration of manganese 0 to 10 ppm. in the solution. Scale for ash weight is 10 × dry weight.

The nutrient solution was used as indicated by CLARK (**6**) and the initial pH was adjusted when necessary to 4.8. Salts were purified by recrystallization as described by CLARK (**8**). The technique followed was altered slightly by substituting Erlenmeyer flasks for beakers, and these were closed with loose plugs of cotton wool. The flasks were placed in a thermostat in which the temperature was kept at 25° C. and exposed to sunshine; no artificial light was used. Controls were kept at all times in the standard solution along with the other flasks, and conclusions drawn from comparison

with these controls. As the light varied at different periods of the year, cultures grown at different times were compared only through the controls.

The stock plants were grown in the inorganic solution free of manganese for several months before being used for the experiments. They had thus passed through some 40 or 50 generations without access to manganese. The number of fronds was counted and the reproduction constant, K, calculated from $\log_{10}N - \log_{10}N_0 = K(t-t_0)$ (**6**). The fronds were dried and the average weight found. The ash and volatile matter were also determined. The method for obtaining the dry weight is somewhat different from that outlined by Su (**16**), who placed the plants for 30 minutes in a vacuum of 5 mm. at 50° C. In this laboratory the plants are dried in micro-cru-

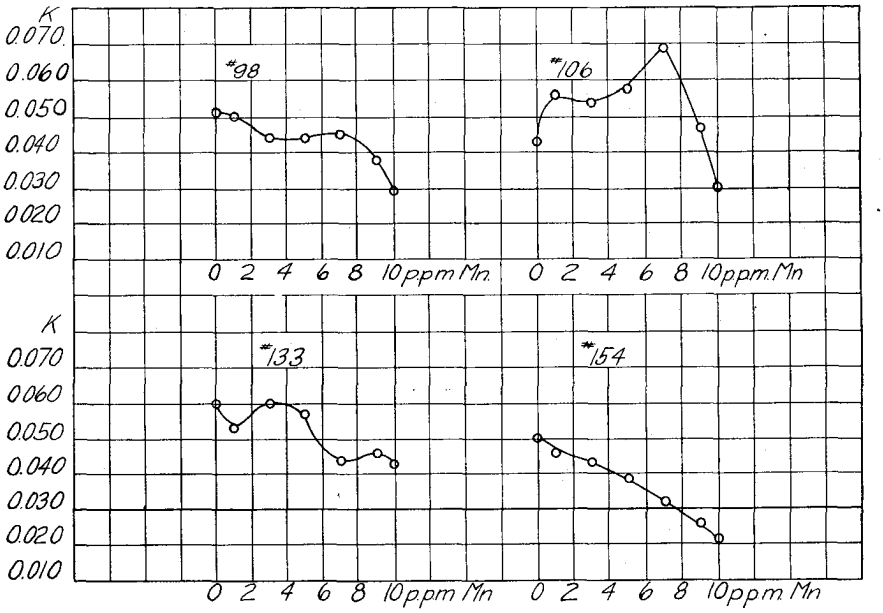

FIG. 2. Reproduction constant, K, for concentration of manganese from 0 to 10 ppm. in the solution.

cibles of platinum under a partial vacuum in a stream of dry air at 45° C. The platinum crucibles were placed in porcelain crucibles in the desiccating chamber, and when the plants were dry (half an hour or over night showed no change in weight) the lids were placed on the porcelain crucibles, and the whole transferred to a sulphuric acid desiccator until room temperature was reached; or the desiccating chamber was allowed to cool and the platinum crucibles transferred to a closed weighing bottle of known weight which was kept in the case of the Ainsworth microbalance (**13**). It was found necessary to have the weighing bottle in the balance. (If kept in the

drying chamber the condensation of moisture during the weighing is very variable.)

Figure 1 shows some typical curves representing the average dry weight and ash content of the fronds both in the medium without manganese and with additions of manganese chloride to make concentrations of that element from 1 to 10 parts per million. It will be seen that there is in every case a rapid rise in both dry weight and ash as the manganese is added until a maximum is reached at about 1 or 2 ppm., after which both are irregular to 10 ppm. Above 10 the manganese concentration becomes increasingly toxic and the plants die off rapidly in the higher concentrations. The rate

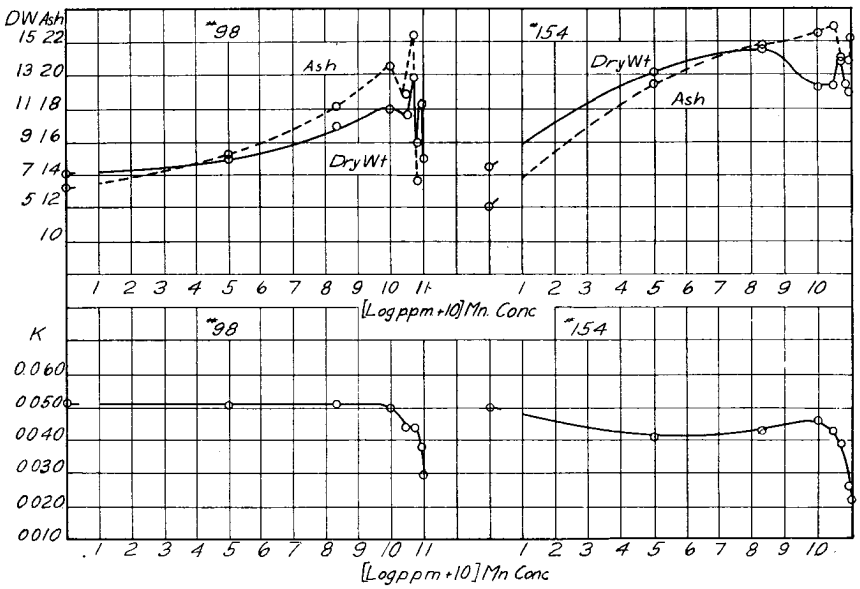

FIG. 3. Average weight and ash, and reproduction constant, K, with logarithm (plus 10) of the ppm. of manganese in the solution, as abscissa. Scale for ash weight is 10 × dry weight.

of reproduction, however, as represented by the constant, K, does not show any regular increase with the addition of manganese. This is shown in figure 2. From 1 to 10 parts per million there is a tendency for K to decrease, and in the region of these concentrations the plants frequently show loss of roots and brown spotting.

That about 1 ppm. of manganese is the concentration for maximum dry weight and maximum ash content under these conditions, is clearly shown in figure 3, where the abscissa is the logarithm of the ppm. When the average of eight or ten sets of weighings was used, the maximum for both dry weight and ash was very close to 1 ppm. No such rise is shown in the repro-

duction constants in figure 3 and the average of eight or ten sets gave an almost horizontal line until close to 1 ppm. The influence of the manganese is therefore probably upon the assimilation of the plant and not upon the reproductive function, at least until the region of toxic concentration is reached.

It was possible to obtain more information concerning the effect on composition, by determining the approximate green weight of the plant. This weight was obtained by washing the fronds through two changes of distilled water, and removing the surface water by pressing lightly between filter papers. The plants were then placed in the platinum crucibles and these weighed inside the closed weighing bottles, as described for the dry weight determination. The green weight showed a close parallel to the dry weight. The difference between the green and dry weights gives a fairly good approximation to the water content, and the volatile matter on ignition is easily obtained. When these were graphed on the logarithm of the concentration of manganese it was seen that the ratio of water, volatile matter and ash varied very little up to 1 ppm. of manganese; on the average there was an indication of a slight tendency for the ash to increase and the water to decrease. From 1 ppm. up, there was the same irregularity with increasing concentration of manganese as shown in the dry weight and ash.

The lower limit of the toxic concentration of manganese under these conditions can be assumed to be somewhere near 1 ppm. Below this concentration the addition of the element to the inorganic salt solution increased both weight and size of plant. It did not, however, have any effect upon the rate of reproduction of the Lemna, neither was there any marked alteration in the ratio of water in the plant to volatile matter or ash. Above 1 ppm. of manganese, reproduction, dry weight, ash weight and composition were all irregular and toxicity increased rapidly with higher concentration.

With regard to the essential nature of manganese we can find no trace of that element in the ash of the plants grown in the inorganic medium unless manganese is added to the solution. The water, after the third distillation (in pyrex glass) is kept in pyrex containers and the plants are grown in pyrex flasks. Boron and silicon may be obtained from this glassware by the plant, and there is a chance that some manganese could be obtained in this way also, although no trace of manganese could be found by evaporating 20 liters of the water which had stood in the glass containers for several weeks; also the ash of the plants grown in the stock solution gave no signs of its presence.

There is some indication that the plant will adapt itself gradually to a concentration of manganese which proves injurious at first; but whether the effect of the manganese, or the element itself, can be carried over for several

generations after the plant goes back to a lower concentration or a manganese free solution, is as yet doubtful, although some results point to that conclusion.

Summary

1. *Lemna major* has been grown in a medium of inorganic salts with and without the addition of manganese.

2. There is no indication that manganese is an essential element in the nutrition of the plant.

3. The size and weight of the fronds increase up to about 1 ppm. of manganese, but the ratio of ash, volatile matter and water is little affected. There is no increase in the rate of reproduction.

4. Above 1 ppm. the concentration becomes gradually more toxic, and reproduction, size, weight and ash all become very irregular.

5. Indications point to the probability of the adaptation of the plant to manganese.

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BIOCHEMICAL RELATIONS BETWEEN IRON AND MANGANESE, AND ORGANIC MATTER IN THE GROWTH OF THE GREEN PLANT

INTRODUCTION AND HISTORICAL

The stimulation of the growth of the green plant by organic matter is generally recognized, although "auximones", which were claimed to be necessary for the growth of plants and to be similar to vitamins for animals, have been shown not to be essentials. This thesis is concerned with experiments carried out in order to investigate further the stimulation of green plants by organic substances. Iron is intimately connected with the production of chlorophyll and it has been stated that manganese affects markedly the utilization of iron by plants; these two elements, therefore, have been studied also in some detail. The plant used in the experiments was Lemna major (Spirodela polyrhiza) which has been cultivated in this laboratory for several years.

Lemna has been used by a number of investigators in the past twenty years. Bottomley (1914, 1917, 1920) and Mockeridge (1920), using Detmer's and later Knop's solution, were unable to get continued normal growth of Lemna unless small amounts of organic extracts were added. They concluded that organic matter in small amounts was essential for the

growth of green plants. To these unknown "essential" substances in the organic extracts they gave the name "auximones." Clark (1924) and Clark and Roller (1924) developed an inorganic media in which Lemna would grow normally without added organic matter. Saeger (1925), Wolfe (1926) and Ashby (1929) confirmed these results - Saeger using a diluted Knop's solution and Lemna major, Wolfe using Shive's solution and Lemna major, and Ashby using Clark's solution and Lemna minor.

Organic matter, therefore, while stimulating the growth of green plants in water cultures is not essential for them. The stimulative effect has been reported by several investigators: Livingston (1907), Schreiner and Skinner (1912), Bottomley (1914), Mockeridge (1920, 1924), Clark and Roller (1924, 1931), Ashby (1929) and Olsen (1930). Ashby considered the action of the organic matter to be of a catalytic nature.

Clark (1930) and Clark and Roller (1931), finding that with certain organic extracts bacterial contamination was difficult to prevent and suspecting that this might influence the results, attempted to obtain sterile cultures of Lemna major. This they finally succeeded in doing. Organic matter was found not to stimulate the growth of Lemna in sterile cultures; the growth rate was always faster in the sterile inorganic medium. Saeger (1930) later succeeded in sterilizing the resting buds of Lemna major and reported,

from a preliminary experiment, some stimulation in the sterile cultures with autolyzed yeast extract.

Both in the sterile and non-sterile inorganic solutions the plants tend to become chlorotic unless the media is changed frequently. Saeger found that putting iron wire in the flasks before sterilization prevented chlorosis. Olsen (1930) has suggested that the beneficial effect of organic matter on the plant is due to its effect upon the solubility of iron. He found that ferric citrate allowed normal growth of plants in alkaline solutions which would precipitate inorganic iron readily. A number of investigators have reported that organic iron compounds seem to be more available than inorganic ones to plants grown in nutrient solutions. Since iron deficiencies frequently occur in highly alkaline soils, low in organic matter, this would seem a logical explanation of one effect of the organic matter in soils on the growth of the green plant. This, however, can only be determined with certainty by a study of nutrient solutions as these are much simpler than the very complicated soil systems.

Little was known of the growth of plants in nutrient solutions until the last part of the 19th Century. Siam-Horstman (1856) grew plants in sand cultures and established the ten elements recognized as essential for the growth of plants. He also added manganese, silicon and chlorine but

these were later regarded as non-essential. The original list of the ten elements essential for all plants has been unaltered, although some of the others have been declared essential for certain plants. Thus Johnston, E. S. (1928) shows boron to be essential for tomatoes, while McHargue (1926) claims manganese to be essential for all plants. McHargue advances the theory that manganese is necessary for the formation of chlorophyll and that it stimulates carbon assimilation. That manganese is not essential for the growth of Lemna major (Spirodela polyrhiza) is shown by Clark and Fly (1930) in Part I of this thesis.

It is interesting to group the elements according to the quantity in which they are found in plants, and according to their source and probable function. Carbon, hydrogen and oxygen, which make up about 90 per cent of the dry weight of most plants, are obtained by them almost entirely from the CO_2 of the air and from water. Nitrogen, potassium, phosphorus, calcium, magnesium, and sulphur constitute about 9 per cent of the total dry weight and are supplied from the soil or from fertilizer. The entire fertilizer industry is concerned with these elements. All the other elements, constituting less than 1 per cent of the total weight of the plant, are found in the soil; in rare instances only they must be supplied in fertilizer.

Of the elements not usually supplied in fertilizers, several investigators have worked with iron and manganese. Gile (1911), Gile and Carrero (1920) and Johnson, M. O. (1924) have shown that in certain soils iron became unavailable. Spraying with ferrous sulphate removed this deficiency and allowed normal growth of plants. Lierow (1924), McLean and Gilbert (1925, 1926, and 1928), Bryan (1929), Schreiner (1928) and Skinner (1929) have shown manganese deficiencies in certain highly calcareous soils. Application of $MnSO_4$ in small quantities was necessary before crops could be grown on these soils. Bryan (1929) has shown also some beneficial effects of copper fertilization on certain chlorotic plants of the Florida Everglades.

No attempt will be made to discuss the literature dealing with the functions of the various elements, other than those of iron and manganese, in the nutrition of green plants. Attention is called, however, to their general groupings as discussed in standard texts dealing with plants, and to the unique role played by iron, manganese and the other elements in their class.

Carbon, hydrogen and oxygen form the bulk of the plant; they constitute the basis of such compounds as carbohydrates, fats and lignins and form also (with nitrogen, sulphur and phosphorus) the proteins, nucleo-proteins and

lipins prominent in the vital organs of the plant.

With the exception of the occurrence of magnesium in the chlorophyll nucleus, the role of magnesium, calcium and potassium is not so definite as that of the elements forming the organic structure of the plant. They are, however, known to be essential for normal metabolism and the last two are absorbed in comparatively large quantities from the soil.

Iron is considered by the majority of writers to act as a catalyst in the formation of chlorophyll. Emerson, R. (1929) has been able to regulate the chlorophyll content of Chlorella by varying the iron content of the solution. Hopkins, E. F. (1930) finds that the growth of Chlorella sp., in a nutrient solution containing a constant amount of sodium citrate, increases with the amount of iron added. Leroux and Leroux (1923) show that the percentage of iron in the leaves is greater than in any other part of the plant. Gile and Carrero (1916) reached the conclusion, from their work on the immobility of iron in the plant, that iron is in some organic form in the leaves. They found that when green, normal rice seedlings were transferred to a solution free of iron, the older leaves remained green but the younger ones grew out almost free of color. Chlorotic leaves, touched with a solution of ferrous sulphate, produced the green color only

where the solution came in contact with the leaf. Therefore, it seems that there is no transportation of this element from the older to the younger parts of the plant, although with potassium, nitrogen and phosphorus, such transportation is definitely shown when plants are placed in solutions deficient in them. This same phenomena has been observed occasionally in this laboratory and is discussed in connection with the experiments on iron.

That iron may have functions different from that of stimulating chlorophyll formation is shown by its essential nature for other than chlorophyll bearing organisms. Buchanan, R. E. and Fulmer, E. I. (1930), in their book on the Physiology and Bio-chemistry of Bacteria, list iron as among the essential elements for these organisms. It is assumed to be the N-activatory for nitrogen-fixing organisms, and may furnish an energy source for the so-called iron bacteria. Mundkur (1928) finds solutions containing iron stimulate the growth of fungi to a greater degree than solutions lacking that element. Hoffer and Carr (1920) show that $FeSO_4$, introduced through the inter-nodal cortical tissues of corn, greatly increases the catalase and oxidase activities; at the same time it brings about rapid wilting of the leaves, browning of the internodes and premature death. From a number of experiments, v. Euler, H. and others (1929-1930) conclude that the catalase activity

of green plants follows closely the chlorophyll content. Euler suggests that either these two substances are affected in the same way by biochemical processes or that one is responsible for the other. Zeile and Hellstrom (1930) recently separated from liver extract an iron porphyrin which they claim contains the catalase function of liver. Its catalase activity is 10,000 times that of hemin, and the ratio between the catalase reaction constant and the Fe-porphyrin content is 10^6 times greater than is the case with ordinary Fe-porphyrin complexes. This year (1931) they have isolated from pumpkin cotyledons an iron porphyrin similar to that from horse liver. It appears, therefore, that iron is an essential constituent of this important oxidizing enzyme.

The theories regarding the functions of manganese in the growth of the green plant have been examined in Part I. The investigations reported in this thesis were undertaken to determine some of the effects of organic matter on the availability of iron, and to find out if this availability of iron is the sole explanation of the stimulation of green plants by organic matter when the plants are grown in nutrient solutions. Since it has been observed that manganese affects the availability of iron, the effect of this element on the various cultures was carefully noted and a summary of the findings is presented in the discussion of experiments recorded later.

APPARATUS AND TECHNIQUE

I. APPARATUS

Roller (1931) changed his sterile cultures in a small room which was previously sterilized by spraying with dilute $HgCl_2$ solution. The room was found rather inconvenient to use, and the apparatus, shown in Figure I, was developed in its place. Roller had used this box without the sterile air but had discarded it in favor of the small room. The apparatus consists essentially of a galvanized iron box, 28 inches high by 24 inches wide and 18 inches deep. The upper 18 inches of the front is of glass. Openings, large enough for the arms to operate freely, are made in the metal below the glass and are covered with hinged metal sheets when not in use. A burner with a pilot light is built into the box so that it draws air from the outside. A hood to carry away the fumes is built about six inches above the burner. This arrangement allows easy handling of the flasks. The burner is operated from inside the box and allows the heat to be turned off except when flaming the plugs. Air from the compressed air line is forced through a copper coil heated to redness, through a cooling coil immersed in running water, to a mat of cotton, and finally into the box through a metal tube sealed at the end but with the sides pierced by tiny holes. The entire set of coils for handling the sterile air

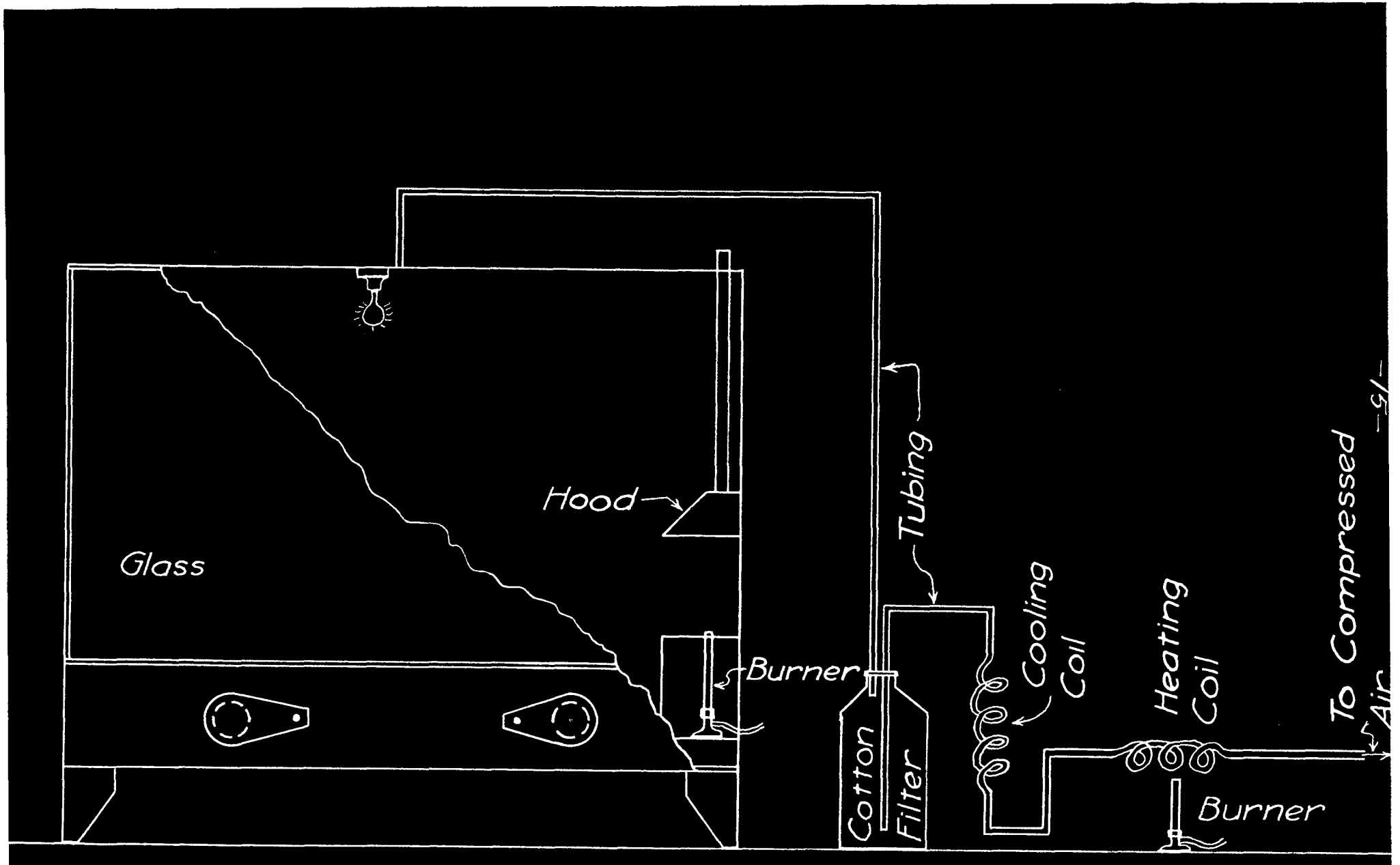


Figure 1 Apparatus for Changing Sterile Cultures

were connected together and sterilized in the autoclave at 21 pounds pressure for 15 minutes, then connected to the box while still hot. The interior of the box is sterilized by removing the glass and spraying with a strong lysol or $HgCl_2$ solution. The sterile air sweeping out through the arm-holes prevents the carrying in of bacteria by movements of the operator. Only five or six contaminations out of about two hundred and fifty changes have been found. It is interesting to note that Saeger (1930) and Hopkins, Wilson and Fred (1931) used similar types of apparatus in their work except that the sterile air feature was lacking.

The Boyce Thompson Institute (1925), Clark (1925), Davis and Hoagland (1928) and Ashby (1928, 1929) have reported apparatus suitable for the growth of plants under controlled conditions of light and temperature. The apparatus used in this investigation is sketched briefly in Figure II. A large double-walled chamber, painted white on the inside, was provided with doors on either side to allow easy access to the plants. A constant temperature bath, regulated by an ether-mercury thermotrol, and having a stirring motor, was placed near enough to the lights to give about 400 candle power light intensity at the surface of the solutions. The light was found to vary only slightly in different parts of the thermostat. The writer wishes to express his thanks to Dr. Kunerth of the

Physics Department who measured the light intensities with a Macbeth illuminometer.

To carry away the heat generated by the lights air was drawn, from an opening near the bottom of the chamber, through a series of cooling coils placed below the constant temperature bath and blown out near the top of the chamber. To prevent excessive heating of the water in the bath when the lights were on, a series of cooling coils were placed in the bath and the flow of water operated by an electromagnet in connection with the ether-mercury regulator. When the temperature dropped below that required, the cooling water was diverted to the drain; when the temperature rose above, the water was started through the coils again. This regulating device is described by Daniels (1929). The temperature was kept at approximately 25°C with a variation of about 0.1°C. A constant temperature bath of the same type was kept in a south window for observing the growth in sunlight. The temperature fluctuated somewhat more than in the Constant Light and Temperature Apparatus. The plants which were kept in the dark were placed in a well insulated box and the temperature maintained at 25°C.

The stock solutions were made from C. P. salts which had been recrystallized several times, and were kept in a modified Shive's apparatus. All glassware used in this work

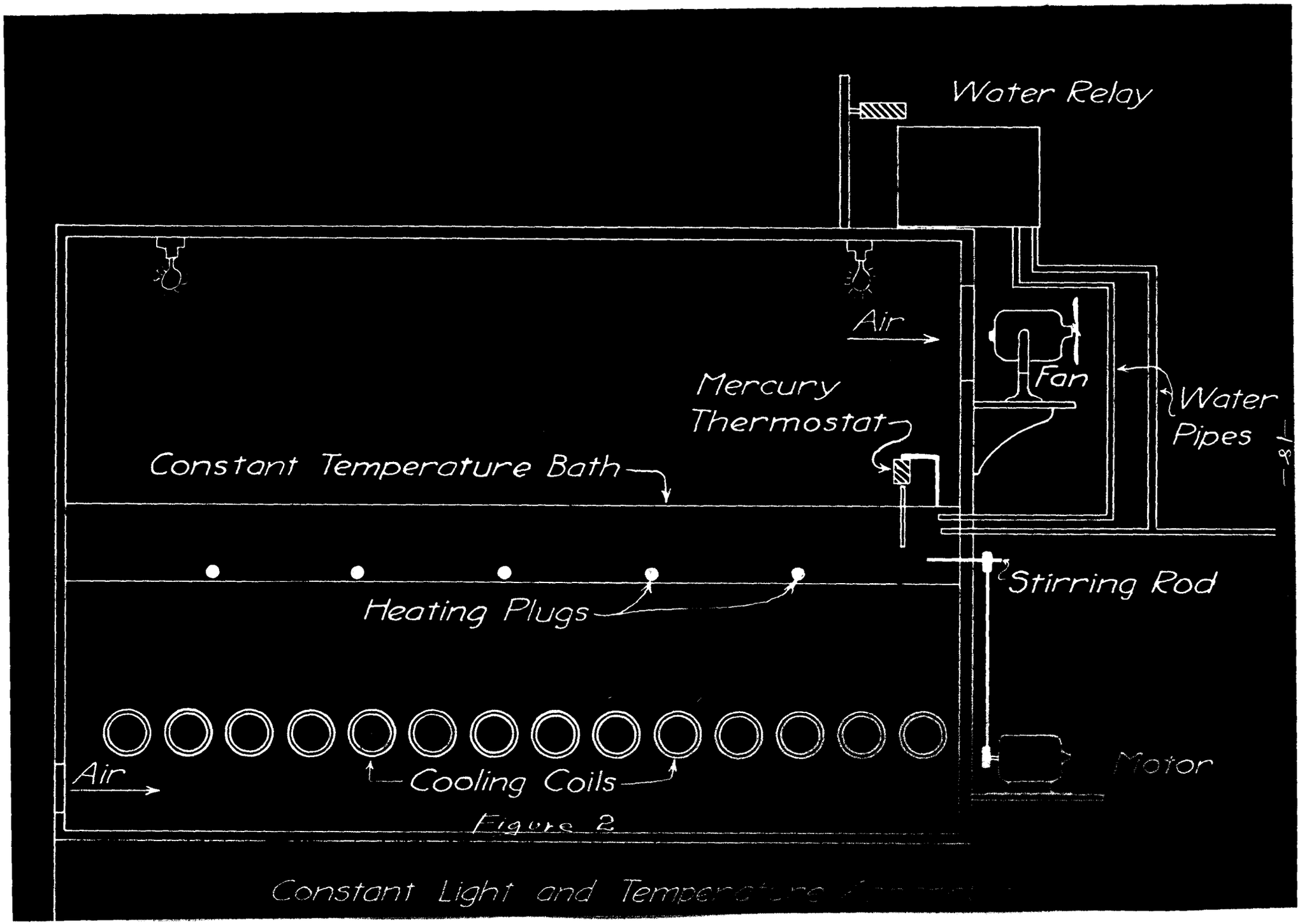

Water Relay
Air
Fan
Water Pipes
Mercury Thermostat
Constant Temperature Bath
Heating Plugs
Stirring Rod
Air
Cooling Coils
Motor

Figure 2

TABLE NO. I

COMPOSITION OF STANDARD SOLUTION

Recrystallized Salts Used	Composition of Stock Solution: cc of stock solution used per 125 cc of nutrient solution	Gms. of salt in 2 liters	Gms. of salt in 1 cc	Moles of salt per liter	Gms. of element per cc. (metal)	Composition of Nutrient Solution: Moles of element per liter (metal)	Mgms. of element per liter (metal)
$CaH_4(PO_4)_2.H_2O$	5.0	5.0669	0.00253	0.01	0.0004	0.0004	16.1
KNO_3	5.0	40.444	0.02022	0.20	0.0070	0.008	312.8
$MgSO_4.7H_2O$	5.0	12.324	0.00616	0.025	0.0006	0.001	24.0
$FeCl_3.6H_2O$	5.0	0.150	0.00007	---	------	-----	0.62

was of pyrex. A specially designed pyrex still was used for the final distillation of water, which had been previously distilled through an ordinary copper still, and then, after treatment with alkaline permanganate, through a block-tin still used for obtaining conductivity water.

The nutrient solution employed in this investigation was that described by Clark and Roller (1924) in their work on auximones. A pH of 4.8, suggested by Clark (1926) as being best suited for Lemna major, was maintained in the check cultures. Table I presents in detail the concentrations of the stock solutions and the composition of the nutrient solution. In the tables summarizing the various experiments, the pH, the source and concentration of iron and the concentration of manganese in each culture are given. The pH values were checked frequently by means of the quinhydrone apparatus described by Clark and Collins (1927). The methods used for adjusting the pH when varying amounts of ferric citrate are added to the solution are given under Organic iron, Part V.

II. TECHNIQUE

The plants were grown in 300 cc Erlenmeyer flasks which were plugged to keep out dust and other contamination. The non-sterile cultures were changed every three days. In some of the experiments on organic iron it was found necessary to change solutions every two days to keep down algae growth. The sterile cultures were changed about twice a week. When plants increased to about fifty in each flask the number was reduced in order to prevent overcrowding. Data was obtained on the rate of reproduction and comparisons were made of the size, color, and appearance of roots, fronds and buds. The solutions were sterilized by autoclaving at 21 pounds of steam pressure for 15 minutes.

The method of obtaining sterile cultures was that developed by Clark and Roller (1931). It is interesting that two other investigators reported similar methods of sterilizing plants, at about the same time. Hopkins, Fred and Wilson (1931) sterilized the seed of leguminous plants by treating them first with a dilute mercuric chloride solution under reduced pressure and finally with Dakin's solution. The apparatus for changing the plants to fresh media was similar to that described here (See "Apparatus"). Saeger, who published an article on the sterilization of Lemna, September, 1930, was well aware of the success of Clark and Roller in

sterilizing the vegetative fronds of Lemna major as he had seen the sterile cultures while visiting here in December, 1929. In his paper he ignored this work, however, as well as a previous publication of Clark (March, 1930) reporting the successful sterilization of Lemna. While Saeger suggests that "sterilization of the entire plants in the vegetative condition is not readily accomplished" this method has been used in these laboratories for two years with marked success. Full-grown sterile vegetative fronds can be obtained readily within a few weeks. Saeger was unable to sterilize the vegetative plants but was successful with the resting buds.

The plants were transferred to fresh media by the use of the apparatus described on pages 14 to 16. At frequent intervals the sterility of the cultures was checked by plating out the fronds on a bacto-peptone glucose agar found by Clark and Roller to be superior to other media tried for this purpose. Very few contaminations were found; attention is called to these in presenting the experimental data.

INORGANIC IRON

In most of the early work on culture solutions inorganic sources of iron were used. If the plants showed a tendency to become chlorotic fresh iron was supplied at intervals or else the solutions were changed frequently. Gile and Carrero (1916) used ferric citrate and ferric tartrate in some studies on the assimilation of iron by rice plants. In recent years a number of investigators have used organic iron compounds for nutrient solution work.

The literature is very confusing with regard to the actual effect of the various iron compounds in nutrient solutions. The value of any iron source, whether inorganic or organic, depends upon the nutrient solution employed and the plant studied. Gile and Carrero (1916) show from their work that colloidal $Fe(OH)_3$, while more available in an acid than in a neutral medium, did not furnish sufficient iron for the growth of the rice plants. $FeSO_4$ was better than $FeCl_3$ in neutral and alkaline solutions but they were of about the same value in acid solutions. Gines (1930) reports normal growth of rice plants in solutions containing $K_3Fe(CN)_6$, $FeCl_3$ or $FePO_4$, but inferior growth with $Fe(NO_3)_3$, $Fe_2(NH_4)_2(SO_4)_4 \cdot 24H_2O$ and $FeSO_4$. Clark (1925) finds that the chloride, phosphate and nitrate give good growth of Lemna major, with the chloride slightly superior to the other two. Deuber (1926)

showed that Lemna may make fair growth on $K_4Fe(CN)_6$. He found that the insoluble $Fe_4(Fe(CN)_6)_3$ was a satisfactory source of iron for soybeans at a pH of 5 but not at a pH of 6. Livingston (1919) suggested precipitated $FePO_4$ as a standard source of iron for culture solutions. Tottingham and Raukin (1923) reported that $FePO_4$ is relatively unavailable to wheat plants in their solutions. Marsh (1922), however, found that with wheat grown in Shive's solution, $FePO_4$ was superior to several organic sources of iron. Sidorin (1914) suggested that the chlorosis of plants grown in alkaline nutrient solutions was due to the precipitation of $FePO_4$.

The adjustment of the hydrogen ion concentration of nutrient solutions is undoubtedly a factor affecting the availability of the iron to the plants. The pH that is most favorable to growth depends upon the nutrient solution employed, the source and concentration of iron used and the kind of plant grown. Clark (1926) found that a pH range of 4.6 to 4.8 was best for Lemna major when $FeCl_3$ was used as the source of iron in his solutions. Deuber (1926) found that with Knop's solution, and $K_4Fe(CN)_6$ as the iron source, the optimum range for Lemna major was 6.2 to 6.8. These plants grow well in a soil solution having a pH of 6.3. Gile and Carrero find that rice will grow in acid solution when $FeSO_4$ is supplied at the rate of 2 parts per million (p.p.m.) while 8 p.p.m. is necessary

to obtain growth in neutral solutions. Tottingham secured better growth with wheat, in a solution having a pH of 4, with 2 p. p. m. of iron, as $Fe_2(SO_4)_3$, than with 10 p. p. m. The interpretation of studies on the availability of iron is confused by the failure of a number of investigators to state the pH of their solutions. It is generally agreed, however, that organic iron compounds are more available than the inorganic ones in neutral or alkaline solutions.

The question of the form in which iron is absorbed by plants has been a subject of considerable controversy. Comber (1922-24) insists that the plants must absorb colloidal iron in neutral and alkaline solutions for no traces of iron ions can be found under these conditions. Olsen (1930) thinks that the plants absorb complex organic iron molecules when grown in alkaline solutions. Thomas (1929) disagrees and quotes the work of a number of investigators to show that a trace of iron ions, insufficient to give a qualitative test, may furnish sufficient iron for the normal growth of plants. He suggests that the equilibria existing between the various forms of an element in contact with its solution necessitate the presence of ions in the solution regardless of the conditions. The equilibria between precipitate, or colloidal gel, and colloidal sol, between sol and dissolved molecules, and between molecules and ions would be displaced by the removal of ions toward the formation of more ions. The

concentration of ions present in solution at any one time, and the rate at which the equilibrium concentration will be renewed when ions are removed by the plant, will depend upon the nature of the material and the conditions imposed upon it. Thus, Thomas presents evidence that silica gel, insoluble phosphates, and insoluble iron and aluminum compounds are sufficiently soluble to furnish the plant with ions.

In the following experiments on inorganic iron the effects of varying the pH of the solution, the source and concentration of iron, the source of light and the concentration of manganese have been studied.

I. EXPERIMENTAL

Lemna major (Spirodela polyrhiza), which for years has been grown in Clark's nutrient solution in this laboratory was used in this investigation. As previously noted non-sterile cultures were changed every two or three days unless the effect of not changing them was to be observed. The sterile solutions were changed every two or three days during the first part of the experiment and every four or five days during the last part.

The conditions for each experiment are given in detail in Table II and the results in Tables II and III. In the first column of Table II is given the number of the culture; to follow the discussion of the results obtained in these experiments, reference should be made to the number in that table in order to obtain quickly the complete description of any culture.

In the second column is given the source of iron. The $FeCl_3$ used was either recrystallized $FeCl_3.6H_2O$ from C. P. salts or Kahlbaum's Eisenchlorid (fur anal. mit guar., etc.). The $Fe(OH)_3$ was prepared by precipitating the recrystallized chloride with NH_4OH, as described in standard methods for the analysis of iron, and washing free of ammonia. It was then made up to a thick suspension and added to the solutions in such a way as to form a layer completely covering the bottom of the flask. The concentration of iron in milligrams of Fe per liter of nutrient solution is given in column three.

The pH, given in column four, is that to which a solution is adjusted before the plants are placed in it. The determination of the hydrogen ion concentration was made by means of a quinhydrone apparatus as described on page 20. The pH was frequently checked although it was found that it did not vary, when the solutions were made up in the same manner, unless the stock solutions were changed. In the case of the $Fe(OH)_3$ experiments (459 A-D) the pH was that of the nutrient solution before the precipitated iron was added.

The concentration of manganese is given in column five in milligrams per liter of nutrient solution. The salt used was $MnCl_2 \cdot 6H_2O$ which was taken from Baker's analyzed salt and recrystallized several times.

In column six, headed "Treatment of Cultures" attention is called to the conditions under which the plants were grown: Sunlight or artificial light, freedom from other organisms, and any special treatment.

The growth rate constant "K" presented in the last column is calculated from the growth rate curves shown in Figure III. In this figure, a few typical curves, illustrating the method of measuring reproduction are given. The growth rate equation, $K = \frac{\log N_f - \log N_i}{T_f - T_i}$ is used in making the calculations. This is a typical rate of growth equation in which N_f and N_i represent the final and initial counts and $T_f - T_i$ the time intervening between the counts. K is the

slope of the growth rate curve in which the log of the count is plotted against time as the abscissa.

In Table III the culture number, the source of light and the general appearance and behavior of the plants are presented in the same order as in the experiments in Table II.

TABLE NO. II

GROWTH OF LEMNA IN INORGANIC SOLUTIONS

Number of Culture	Source of Iron	Conc. of Iron mgms./L.	pH	Conc. of Mn mgms./L.	Treatment of Cultures (n-s indicates non-sterile) (CLTA indicates Constant Temperature-Light Apparatus)	Growth K x 100
323	$FeCl_3$	0.5	4.8	1.0	non-sterile; grown in sunlight)	6.0
323	"	"	"	1.0	" " " " CLTA	7.0
324	"	"	"	0.0	" " " " sunlight)	6.5
483	"	"	"	0.5	" " " " "	8.5
484	"	"	"	0.2	" " " " "	6.0
485	"	"	"	0.1	" " " " "	7.0
489A	"	"	4.5	1.0	" " " " "	6.3
489B	"	"	5.1	1.0	" " " " "	3.2
489C	"	"	4.5	0.0	" " " " "	7.0
489D	"	"	5.1	0.0	" " " " "	4.0
489E	"	"	4.5	0.2	" " " " "	7.3
589F	"	"	5.1	0.2	" " " " "	4.0
378A	"	"	4.8	1.0	n-s CLTA(0.13 gm. Fe wire	2.2
378B	Iron wire		4.8	1.0	" " " " "	1.2
378C	Ferric Citrate	0.5	4.8	1.0	" " " " "	5.5
445A	$FeCl_3$	5.0	4.8	1.0	" sunlight	6.5
445B	"	5.0	4.8	1.0	" CLTA	8.0
459A	$Fe(OH)_3$	precipitate	4.6	0.0	" " (fresh $Fe(OH)_3$ layer	5.2
459B	"	"	4.8	0.0	" " " " "	4.7
459C	"	"	4.6	0.0	" sunlight " "	4.5
459D	"	"	4.8	0.0	" " " "	3.0

TABLE NO. II (continued)

GROWTH OF LEMNA IN INORGANIC SOLUTIONS

Number of Culture	Source of Iron	Conc. of Iron mgms./L.	pH	Conc.of Mn mgms./L.	Treatment of Cultures (n-s indicates non-sterile) (CLTA indicates Constant Temperature-Light Apparatus)	Growth K x 100
527	$FeCl_3$	0.5	4.8	0.0	CLTA sterile soln;n-s plant	5.0
528	"	"	"	1.0	" " " " "	4.0
529	"	"	4.5	0.2	" " " " "	6.4
530	"	5.0	4.8	1.0	" " " " "	5.3
531	"	"	4.5	0.2	" " " " "	7.1
532	"	"	4.5	0.0	" " " " "	7.0
556G	"	0.5	4.8	0.0	sterile; CLTA----------	8.3
556H	"	"	"	1.0	" " ----------	7.0
556I	"	"	4.5	0.2	" " ----------	7.4
556J	"	5.0	"	0.2	" " ----------	8.0
759A	"	0.5	4.8	0.0	sterile cultures grown in CLTA)	8.2
759B	"	"	"	0.5	" " " " "	7.5
759C	"	"	"	0.2	" " " " "	8.9
759D	"	"	"	0.02	" " " " "	8.8
759E	"	"	"	2.0	" " " " "	7.0
159F	"	"	"	4.0	" " " " "	7.9
775A	"	5.0	"	0.0	" " " " "	9.7
775B	"	"	"	4.0	" " " " "	10.5
775C	"	"	"	2.0	" " " " "	6.9
775D	"	"	"	0.5	" " " " "	7.6
775E	"	"	"	0.2	" " " " "	7.7
775F	"	"	"	0.02	" " " " "	9.2
844	"	0.5	"	0.2	sterile;CLTA; iron wire; (9 cc $CaH_4(PO_4)_2$	8.6

TABLE NO. II (continued)

GROWTH OF LEMNA IN INORGANIC SOLUTIONS

Number of Culture	Source of Iron	Conc. of Iron mgms./L.	pH	Conc. of Mn mgms./L.	Treatment of Cultures (n-s indicates non-sterile) (CLTA indicates Constant Temperature-Light Apparatus)	Growth K x 100
365A	$FeSO_4$	16.0	6.3	1.0	n-s, CLTA, plus 60 ppm citric acid)	6.7
365B	$Fe_2(SO_4)_3$	"	"	"	" " " " " " "	5.0
365C	$FeSO_4$	"	"	"	" " " 28.5 ppm glucose)	5.7
365D	$Fe_2(SO_4)_3$	"	"	"	" " " " " "	6.0
365E	$FeSO_4$	"	"	"	" "	5.7
365F	Ferric Citrate	"	"	"	" "	8.7

TABLE III

DESCRIPTION OF THE APPEARANCE AND BEHAVIOR OF LEMNA IN INORGANIC CULTURE SOLUTIONS

Number of Culture	Source of Light	Description
323	sunlight	medium size, thin, short roots, medium green color, red tinge.
323	CLTA	" " " " " pale " " slight tinge.
324	"	" " " " " medium " " " "
483	sunlight	gradually became smaller, lost color.
484	"	medium size, but slow loss of color after some time.
485	"	gradually became smaller and lost some color but not as much as 483.
489A	"	quickly lost size, color and some roots, then remained small, pale green.
589B	"	" " " " " " " plants died after two weeks.
589C	"	became smaller but retained a fair color, lost some roots.
589D	"	" " yellow and lost roots.
589E	"	remained medium size and pale green color.
589F	"	became small, yellowish and died after two weeks.
378A	CLTA	became contaminated with algae and died in a short time.
378B	"	" " " " " " " " " "
378C	"	medium size, roundish, medium green, reddish tinge.
445A	sunlight	medium size, pale green color, thin, short roots, reddish tinge.
445B	CLTA	medium size, yellowish green.
459A	"	" " " "
459B	"	smaller than 459A, with less color.
459C	sunlight	gradually became smaller, lost roots and turned yellow.
459D	"	became as 459C but in shorter time.

TABLE III (continued)

DESCRIPTION OF THE APPEARANCE AND BEHAVIOR OF LEMNA IN INORGANIC CULTURE SOLUTIONS

Number of Culture	Source of Light	Description
527	CLTA	large with normal green color for two weeks then became smaller.
528	"	became quickly smaller and chlorotic; dead in two weeks.
529	"	large and normal green color throughout period studied (25 days).
530	"	medium to small in size; yellow-green.
531	"	large and green.
532	"	" " "
556G	"	medium size and slightly less green than normal.
556H	"	medium size but gradually becoming chlorotic.
556I	"	medium size between 556G and 556H in color.
556J	"	larger and greener than others in this series.
759A	"	medium size with slightly less than normal green color.
759B	"	small; yellow-green; lost roots.
759C	"	medium size but yellow-green.
759D	"	larger and greener than ones above.
759E	"	about same size as 759D but with slightly less color.
759F	"	smaller, with less color then 759E.
775A	"	large and with slightly less than normal green color.
775B	"	medium size but yellow-green with tendency to lose roots.
775C	"	" " " " " " " " " "
775D	"	medium size, green.
775E	"	" " "
775F	"	larger and greener than 775D and 775E;depth of color deepend with less Mn.
844	"	slightly less than normal size, green but lost roots.

TABLE III (continued)

DESCRIPTION OF THE APPEARANCE AND BEHAVIOR OF LEMNA IN INORGANIC CULTURE SOLUTIONS

Number of Culture	Source of Light	Description
365A	CLTA	large and green but with tendency to lose roots;algae quite abundant.
365B	"	similar to 365A but lost roots more easily and was more infested with algae.
365C	"	large and green for few days, then became small and chlorotic; algae abundant.
365D	"	medium to small; yellow and badly infested with algae.
365E	"	medium to small; yellow-green.
365F	"	large;green; algae abundant.

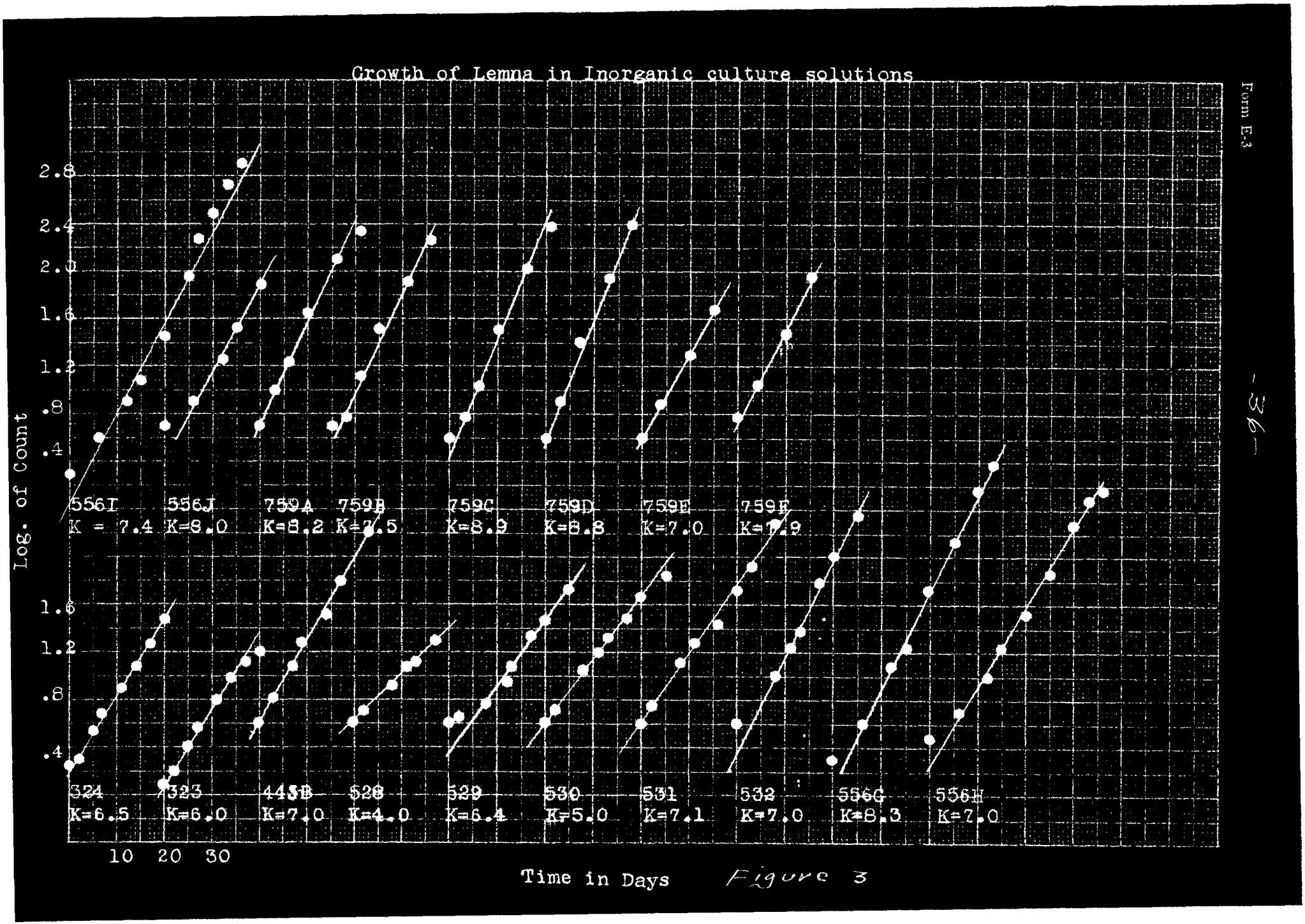

Growth of Lemna in Inorganic culture solutions
Log. of Count
2.8
2.4
2.0
1.6
1.2
.8
.4
556I K = 7.4
556J K=8.0
759A K=8.2
759B K=7.5
759C K=8.9
759D K=8.8
759E K=7.0
759F K=7.9
1.6
1.2
.8
.4
324 K=6.5
323 K=6.0
443B K=7.0
528 K=4.0
529 K=6.4
530 K=5.0
531 K=7.1
532 K=7.0
556G K=8.3
556H K=7.0
10 20 30
Time in Days
Form E-3
-36-

Figure 3

II. DISCUSSION OF EXPERIMENTAL RESULTS

In the check of the optimum pH range the three values selected were 4.5, 4.8, and 5.1, which represent the optimum pH for this solution, as found by Clark (1926), and points on either side of the optimum. With few exceptions the higher rate of growth was in the solutions having the lower pH. It must be kept in mind in interpreting these results that Clark changed his solutions daily and used a light intensity twice that used here. These solutions were changed every three days.

If the cultures (323, 324, 483-485 and 489A-F) are arranged in groups of corresponding manganese content the growth rates for the pH values 4.5, 4.8 and 5.1 are, respectively: (1 p.p.m. Mn) 6.3, 6.0 and 3.2; (0.2 p.p.m of Mn) 7.3, 6.0 and 4.0, and (with no Mn) 7.0, 6.5 and 4.0. There is very little difference, therefore, in the growth rates obtained at pH 4.5 and pH 4.8, with a slight advantage for pH 4.5; the more alkaline solution is distinctly unfavorable for growth.

The same relationship is noted in the case of the cultures (459A-D) receiving precipitated iron, the more acid solutions producing a higher growth rate in both sunlight and artificial light. $Fe(OH)_3$ was distinctly inferior to $FeCl_3$ as a source of iron for Lemna; the plants not only grew at a slower rate but were small and chlorotic. Iron wire (378 A-C)

proved to be toxic even in the presence of other sources of iron. The toxicity was removed by changing to sterile conditions and the plants grew well for some time. Later they became stunted, probably due to the precipitation of the phosphorus as $FePO_4$. Saeger (1930) has shown iron wire to prevent chlorosis in sterile cultures of Lemna with a dilute Knop's solution.

When the concentration of iron (added as $FeCl_3$) was increased 10-fold, i.e., to 5 mgms./liter, more rapid reproduction, and in most cases larger and greener plants, were obtained. If these two concentrations are compared when other conditions are the same, for 0.5 and 5.0 mgms. respectively, the following growth rates are obtained: (323, 445a) 6.0 and 6.5, (323, 445B) 7.0 and 8.0, (429, 531) 6.4 and 7.1, (556I, 556J) 7.4 and 8.0, (759A, 775A) 8.2 and 9.7, (759D, 775F) 8.8 and 9.2, (759B, 775D) 7.5 and 7.6, (759F, 775B) 7.9 and 10.5, (759C, 775E) 8.9 and 7.7 and (759E, 775C) 7.0 and 6.9. Thus out of ten sets of experiments performed under varying conditions of light, pH, manganese content and sterility, the higher concentration of iron produced the higher growth rate in all but two cases.

The effect of manganese was noted in each of these experiments. Since it had been found in the previous work on manganese (Part I) that, with varying amounts of manganese, there was very little effect on the growth rate of Lemna until toxic concentrations were reached, experiments were performed

to note the effect of this element at concentrations slightly above and below the point (1 p.p.m.) previously observed as the lower limit of toxicity. None of these points was studied in the first paper.

When experiments, which are otherwise similar, are arranged in order of decreasing concentrations of manganese, the K is found as follows: (323, 483, 484, 485, 324) 6.0, 8.5, 6.0, 7.5, and 6.5; (489A, 489E, 489C) 6.3, 7.3, and 7.0; (489B, 489F, 489D) 3.2, 4.0 and 4.0; (528, 527) 4.0 and 5.0; (530, 531, 532) 5.3, 7.1 and 7.0; (556H, 556G) 7.0 and 8.3; (759A-F) 7.9, 7.0, 7.5, 8.9, 8.8 and 8.2; (775A-F) 10.5, 6.9, 7.6, 7.7, 9.2, and 9.7.

From these experiments it will be seen that in the sterile cultures (759A-F), (775A-F) the highest concentrations of manganese gave, for the duration of the experiment, marked stimulation of the growth rate but the plants became rapidly smaller in size and lost color and roots. In general the plants in solutions containing no manganese grew as rapidly as plants in solutions containing an optimum concentration of this element. Concentrations of manganese around 1 p.p.m. and above were toxic, causing loss of color, decrease in size and loss of roots. The lower concentrations produced plants somewhat larger than those grown in the absence of manganese. The conclusions, with respect to manganese, reached in Part I of

this thesis, are, therefore, found to be true when such conditions as pH, source of light and concentration of iron are varied. It is noted that manganese tends to sensitize the plants to conditions which are unfavorable to growth. If the plants do poorly in a solution because of unfavorable hydrogen concentration or too strong light, plants in the same solutions containing manganese will be first to show the abnormal condition. Under the artificial light the toxic effect of manganese was found to be greatly increased, the plants becoming chlorotic much quicker than in the sunlight. In all cases the plants grew faster under the artificial light although the solutions containing higher concentrations of manganese, or having an unfavorable pH, produced chlorosis and stunting more quickly than in the sunlight.

It had been found that mono-calcium phosphate, after standing in the laboratory for sometime, became less available to the plants, when placed in solution. Freshly prepared salt was superior to that which had been crystallized for some time. That the phosphate crystals gradually change on standing has been reported by Stoklasa (1899) and Cameron and Seidell (1904). Some preliminary experiments on freshly recrystallized mono-calcium phosphate (Clark, 1931) were therefore carried out. The work was discontinued owing to lack of time but indications were that a concentration corresponding to 22.5 mgms. of

calcium per liter (added as mono-calcium phosphate) gave better growth and greener plants than 16.1 as employed in previous work. Lower concentrations were distinctly unfavorable to reproduction.

Preliminary experiments were also carried out to determine the effect of adding organic substances to solutions containing organic iron upon the availability of that element. The results are given at the end of Tables II and III (365A-F). The organic substances, citric acid and glucose, produced reproduction that was slightly better than $FeSO_4$ alone, but the plants were somewhat larger and greener. It was noted that the same concentration of iron in the form of ferric citrate, and at the same pH, gave much faster reproduction than the other cultures; the plants were at the same time larger and greener. This would show that iron which is already in an organic form is more available than when added to the solution as an inorganic salt with an organic acid present. Hopkins (1930) reports experiments with Chlorella sp. in which increased growth was obtained by adding increasing quantities of sodium citrate to a solution containing a constant quantity of iron, and also by adding increasing quantities of iron to a solution containing a definite quantity of the citrate. He does not report checking this with a solution containing ferric citrate.

Solutions which were sterilized, but with non-sterile plants grown in them, produced slightly lower rates of reproduction than non-sterile solutions, although the appearance of the plants did not deteriorate. Sterile plants in sterile solutions reproduced faster than plants in any of the other cultures. This confirms the work of Clark and Roller (1931) who first used Lemna in sterile culture studies.

From this work and that of other investigators it is evident that when plants are grown in solutions containing, as the source of iron, the salt of a highly ionizable acid, the pH must be kept well to the acid side. The instability of iron compounds in solution is well known. Quarteroli (1915) shows that a concentration of $FeCl_3$ of 0.00054 moles per liter will be 95 per cent hydrolized in about six hours. Since this concentration is 60 times that used in Clark's solution, the concentration of ferric ions would be expected to be very low at the moment of placing the plants in the solution. If the solubility of $Fe(OH)_3$ is exceeded, precipitation of the iron should take place. The per cent solubility of $Fe(OH)_3$ is given by Landolt and others (1923) as ranging from 1.5×10^{-5} to 4.8×10^{-9}. If the iron in Clark's solution were all in the form of $Fe(OH)_3$ the per cent concentration would be 1×10^{-4}, that is, approximately one-tenth of the iron should precipitate if the upper limit given by Landolt is correct. At the lower limit only 1/20,000 of the iron should remain in

solution. Hopkins and Wann (1925) reported that only 1/100 of the iron in a solution of pH 6.0 remained in solution after being allowed to stand over night. Olsen (1930) found that a larger part of the iron added to a Knop's solution precipitated at a pH of 3.5 and not more than 1×10^{-9} mgms./liter remained in solution at a pH of 7.0. To obtain iron from such solutions the plants must be able to absorb the ions from exceedingly low concentrations.

Gile and Carrero (1916), after filtering a nutrient solution in which rice grew normally, found 1×10^{-7} mgms./liter of iron in solution. This is a per cent solubility of $Fe(OH)_3$ of 2×10^{-5}. Hopkins (1930)grew Chlorella in solutions having a concentration of 8×10^{-5}gms. of iron per liter. Sidorin (1925) found that a concentration of 1×10^{-5} mgms./liter would bring back the green color to chlorotic leaves when applied to them directly. In this laboratory no traces of iron were found in the inorganic solutions in which plants had been growing for three days, and the plants would not grow in the filtered solutions.

It appears, therefore, that while most of the iron which is added to a nutrient solution in inorganic form will precipitate out, plants may in some cases obtain enough iron from these very dilute solutions for normal growth. Undoubtedly the pH would be a determining factor for growth in these solutions.

Attention should be called at this point to the difficulty of obtaining accurate analytical data on such small quantities of material and to the apparent inability of investigators to distinguish the ionized, molecular and sol states of iron when the total concentration is very low. Filter paper may absorb an appreciable amount of the iron ions, and would remove some from the sol state as well. Experiments have shown that such reactions as the precipitation of $Fe_4(Fe(CN)_6)_3$ will not take place when a dilute solution of $FeCl_3$ is boiled. The stabilization of iron sols by invert sugar has been reported by Riffard (1874), and the effect of organic acids in preventing the precipitation of iron from solutions is well known in analytical procedures. That plants may secrete these substances in small amounts has been shown; Knudson (1920) has found invert sugar secreted from the roots of peas. The dissolving effect of chloride and nitrate ions on precipitated $Fe(OH)_3$ has been shown by Pickering (1913), Joffe and McLean (1928) and others.

Colloids usually settle very slowly when coagulated and attain final equilibrium with the solution only after some lapse of time. In a nutrient solution supersaturation with iron would exist until all the colloidal material had settled out. The influence of pH, salts in the solution and the presence of plants would alter the rate at which equilibrium in the various phases could be attained. The poor growth,

obtained when plants are placed in solutions to which freshly precipitated iron has been added may be due to the slowness with which the iron dissolves. Placed in the nutrient solutions to which soluble iron compounds are added, the plants would be growing in a medium which is supersaturated with respect to iron, while in the solutions receiving the precipitated hydroxide they would be growing in a medium that is probably quite unsaturated with the element. The frequency with which solutions are changed is shown by Clark (1925) to have marked effect upon the rate of reproduction of Lemna in inorganic cultures.

The work of a number of investigators, therefore, points to the conclusion that the effects of varying the pH, and the concentration and source of iron, upon the growth of green plants may be explained by reactions involving the absorbtion of iron from the solution in an ionized or molecular state. There is evident need for more extensive study of the reactions occurring in nutrient solutions, especially in regard to the development of technique for studying the equilibria involved in very dilute solutions.

The effect of manganese noted in these experiments bears out the results obtained in the previous work (Part I). Manganese does not affect appreciably the reproduction rate of Lemna major, except in the toxic range of 1 p.p.m. and above.

It is not essential for the growth of Lemna. Generally the lower concentrations produced about the same rates of reproduction as were produced when manganese was absent; the plants were somewhat larger and greener. With truck crops such as studied by Schreiner (1928), Skinner (1929), Gilbert and McLean (1925, 1926, 1928), and others, the increased yields of fruit and seed obtained on adding manganese may be explained by the beneficial effect upon the vegetative portion of the plant.

The majority of workers who have studied the effects of manganese on the growth of plants report stimulation of the size and the depth of color of the vegetative portions; toxic quantities caused stunting and chlorosis. This would appear to be a stimulation of the photosynthetic activities of the plant. McHargue (1926) claims manganese is necessary for chlorophyll formation. While the stimulation effect seems to be independent of the iron concentration, the plants in solutions containing manganese become chlorotic more quickly when the pH is increased or the light intensity increased, which indicates that the effect is on the availability of iron. That manganese-induced chlorosis generally occurs on highly calcareous soils, low in organic matter, would bear out this conclusion. Spraying with ferrous sulphate is a standard treatment for this type of chlorosis. Rippel (1923) found that the addition of iron removed chlorosis of barley

caused by the presence of manganese in the nutrient solution; as the iron content of all plants was the same, he concluded that the effect of manganese was upon the action of iron in the tissue but not upon its assimilation. Hopkins (1930), from some experiments on Chlorella sp., concluded that manganese tends to keep the iron in the plant oxidized to the ferric state and that the growth of the plant depends upon a proper ratio between the concentrations of iron and manganese present in the tissue. It has been shown here that the ratio of the manganese and iron concentrations in the nutrient solution has no relation to the toxicity of the manganese. This point will be further brought out in the experiments on organic iron.

ORGANIC IRON

The growing partiality for organic iron compounds as sources of this element for nutrient solution studies is well known. Iron in this form is generally found more available to green plants; the availability is not affected by the reaction of the medium to the same extent as that of inorganic iron. That the stimulation of the growth of green plants, in nutrient solutions may be due to the effect of the organic matter on the availability of iron is a matter of controversy at the present time. Work was undertaken here to study the behavior of organic iron when used in nutrient cultures. The technique was employed as in the previous work on inorganic iron.

Organic matter in soils has been shown to affect the availability of iron to plants. Gile (1911) and Gile and Carrero (1920) found iron chlorosis occurring on highly calcareous soils which were low in organic matter. Soils high in organic matter did not show iron deficiencies even though the lime content was high. Johnson, M. O., in a series of papers (1917, 1924) on the chlorosis of pineapples, ascribes this effect to the presence in the soil of a high MnO_2 content and a near-neutral or alkaline condition of the soil. The iron is kept in an oxidized state and is precipitated under the conditions found. Joffe and McLean (1928) could find no iron

present in soil solutions except under conditions of very high acidity. Iron and aluminum sols of various degrees of dispersion were reported in soils having a pH of 4.0 or below. Where nitrate and chloride ions were present iron sols existed in soils with a pH as high as 5.4. Gile and Carrero, however, show that plants can obtain sufficient iron in a soil from which the water extract gives no trace of this element.

Olsen (1930) states that the benefit of organic matter on soils as well as in nutrient solutions may be explained by the absorbtion of the iron, by the plant, in the form of a complex ion. He found that humus extract stimulated the growth of Lemna in culture solutions containing $FeCl_3$, but not in solutions containing ferric citrate as the iron source. Carrero and Gile (1916), Marsh (1922, 1923), Tottingham and Raukin (1923), Hopkins and Wann (1925), and Hopkins (1930), among others, have shown that in nutrient solutions organic iron sources are superior to inorganic. Gile and Carrero (1920), however, found that pure organic iron compounds were poor sources of iron for rice when added to a soil showing lime-induced chlorosis. Barnyard manure and substances of that nature removed the iron deficiency.

The form in which the iron is absorbed by the

plants from organic iron sources is debatable. The theory of Olsen (1930) favoring absorbtion of complex organic ions, the theory of Comber (1922), suggesting the intake of colloidal particles, and the colloid-solution equilibrium theory of Thomas (1929) are discussed in the work on inorganic iron, pages 25 and 26. Hopkins (1930) found, by conductivity and freezing point determinations, that sodium citrate depressed the ionization of $FeCl_3$ until a point was reached where the ratio of citrate salt to $FeCl_3$ was 1:1.5. At this point the solution gave no tests for iron and behaved like a true compound. He was able to prepare a potassium iron citrate of definite composition but could not crystallize it. Though no test for ferric ions could be found on redissolving the precipitated material, strong tests for ferrous ions were obtained. He suggests that the iron in organic combination is ionized sufficiently to furnish iron ions to the plant, but is not precipitated by alkalies. Olsen (1930) claims also that ferric citrate is not precipitated from solution by alkalies. These investigations indicate that the action of organic iron is that of a soluble organic complex which is not precipitated in neutral or alkaline solutions and which, by slow ionization of the complex ion (iron is shown to be present in the acid radical), releases sufficient iron for use by the plant.

A number of other investigators, however, have shown that organic iron compounds are precipitated from nutrient solutions on standing and that this is true especially when the solutions are placed in the light. Marsh (1922) and Reed and Haas (1924) report the precipitation of iron from organic compounds when placed in nutrient solutions. Todd (1927) showed that iron and ammonium citrates tend to precipitate in the presence of calcium and magnesium ions. Hopkins (1925) found that sodium citrate prevented the precipitation of iron in a nutrient solution at a pH of 7, only when the calcium content was very low. Harrer (1929), Scoville (1915), Winther and Oxholt-Howe (1914), Fry and Germe (1928) and Burt (1928) present evidence that organic iron compounds are very sensitive to light of various sources and undergo reduction of the iron to a ferrous condition with the formation of such compounds as acetone, acetone dicarbonic acid and CO_2. Nuccuroni (1920) showed that citric acid when added to soil is decomposed into acetone in the presence of iron salts and iron containing fertilizers.

In the following work which was carried out on organic iron, ferric citrate was used as typical of this class of compounds. Preliminary studies on the properties of ferric citrate in nutrient solutions were carried out prior to the investigations of its effect upon the growth of Lemna. Attempts to prepare a ferric citrate of definite composition

were made, and the results of that work are presented at the end of the experiments on organic iron.

ORGANIC IRON

I. PRELIMINARY EXPERIMENTS ON THE BEHAVIOR OF FERRIC CITRATE IN SOLUTION.

Prior to the plant culture experiments, a study of some of the properties of ferric citrate was made. A brief survey of the work, and a discussion of some of the results obtained is presented here.

Ferric citrate (U.S.P.) is a brown-red non-crystalline substance and occurs in small nuggets which show conchoidal fracture and deepen in color with the fineness of division. It is very slowly soluble at 50°C but dissolves much more rapidly when stirred at 70°C. Occasionally, at the higher temperature, a yellow mud which is practically insoluble, precipitates. Fifteen grams of the U.S.P. salt will dissolve in about 500 cc of solution. After the material is once dissolved it may be evaporated entirely to dryness without giving any evidence of reprecipitation. If evaporated in a shallow dish at 50°C, red laminas are obtained. These appear to dissolve more readily than the nuggets. If the concentrated syrup is treated with an equal volume of alcohol a thick, yellow mud is thrown down which, if filtered, washed, and dried at 50°C, may be obtained as a yellow powder. Before drying this substance will dissolve quite readily but only slightly faster than the laminas after drying. If the fresh mud is redissolved and evaporated to

dryness in a shallow dish, red laminas are obtained. These are more soluble than those obtained from evaporating a solution of the ordinary U.S.P. salt. The compound showed no tendency to crystallize under any conditions.

Solutions of ferric citrate are yellow-green when first made up, but if allowed to stand in the light gradually take on a reddish tinge. A solution placed in a light of 400 candle power intensity and allowed to stand for several days turned to a deep red, opaque liquid but showed no precipitation. Successive autoclaving of a freshly prepared solution deepened the color but did not cause precipitation.

When ferric citrate is used as the source of iron in the nutrient solution described in Table I, the behavior toward light and sterilization is markedly different. When the salt is first dissolved, the color of the solutions containing the higher concentrations of iron, are yellow-green when the solution is acid, and red when the solution is alkaline. When these solutions were placed in the light, precipitation took place. After five days in the light they were filtered and the filtrates examined for iron by the sulphocyanate method described in Scott's "Standard Methods of Analysis" IV Ed., Vol. I, page 261. This test is claimed to be sensitive to 1 part of iron in 50,000,000 parts of solution. The solutions tested represented a range of 16 to 80 mgms. of iron per liter originally present, and a pH range

from 4.8 to 8.8. The iron content was found to be uniformly of the order of 1 p.p.m. and showed no relation with the amount added. Analysis of the precipitates were made by the Zimmerman-Reinhardt method. In solutions containing 10.7 mgms. of iron in 125 cc of the original solution, after five days growth of plants, the average of five analyses showed 10.5 mgms. precipitated and 0.07 mgms. in solution, leaving 0.13 to be accounted for by the plants and by experimental error. Plants would not grow on the solutions which had been filtered.

A colorimetric test for ferrous ions was made on the same solutions using a 1 per cent solution of diisonitrosoacetone which is claimed by Dubsky and Kuras (1929) to be sensitive to 1 part of ferrous ions in 10,000,000 parts of solution. Only a slight trace of color could be noticed in any of the solutions. Fifteen minutes of autoclaving at 21 pounds of steam pressure seemed to precipitate the iron as completely as five days in the light although no analyses were run on these solutions.

Attention should be called to the discussion of the inadequacy of analytical methods for very low quantities of iron (page 44). While the total figures, in the analyses given, check fairly well with the iron originally supplied they do not show in what forms the iron may exist in the solution. It appears, however, that ferric citrate added to Clark's nutrient solution is neither available to the plants

in the form of a soluble complex ion as suggested by Olsen (1930), nor is it supplied by slow ionization of the complex as suggested by Hopkins (1930). In view of the fact that decomposition in light is known to occur with organic iron compounds, the iron must be released first in the ferrous condition. It is slowly reoxidized and goes through the various stages of hydrolysis and coagulation. That this process may be quite slow is shown by Harrer (1929) who found ferric ions present in solution after sixty days.

Tests for ferric and ferrous ions were made on water solutions of ferric citrate. The sulphocyanate method was used for the ferric ions, but $K_3Fe(CN)_6$ was substituted for the diisonitrosoacetone test for ferrous ions. Freshly dissolved U.S.P. ferric citrate gave no test for ferrous ions, but showed a slight test for ferric. The reprecipitated material gave, in the freshly prepared solutions, a strong test for ferric ions and a distinct test for ferrous ions. The concentrations of both ions accumulated slowly when the solution was allowed to stand in the dark. When the ferric citrate solutions were placed in a strong light, rapid reduction of the ferric ions occurred. Almost complete reduction was attained in one to two hours when a solution of the citrate was placed two inches from the light furnished by a water-cooled 200-watt Mazda bulb. These experiments

afford an explanation of the mechanism of the precipitation of iron discussed in the preceding paragraph. In the light the ferric citrate complex is destroyed with the formation of ferrous ions, and possibly acetone and CO_2, as suggested by a number of investigators (page 51). Strong light, alkaline medium and the presence of green plants bring about reoxidation of the iron, with subsequent precipitation as $Fe(OH)_3$, $FePO_4$ and other insoluble compounds. The presence of considerable quantities of ferric phosphate in the precipitate was easily demonstrated.

In preparing the culture solutions a number of concentrations of ferric citrate were used in Clark's media and the pH values determined for each solution. Varying amounts of 0.005 N and 0.2 N KOH were then added to these and the pH redetermined. From a large number of such trials a chart of the buffering power of ferric citrate was made and the amount of KOH which it was necessary to add to a total of 125 cc of solution to attain the desired pH was calculated. A number of the calculated values were checked and corrections made where necessary. Repeated checks of points on the chart have been made from time to time but without altering the results which are shown in Figure IV, page 60.

Table IV gives the experimental data from which the curves are derived. It is noticed that with increasing pH the relative amounts of KOH (the cc of KOH in a liter of solution

per milligram of iron per liter) increase, i.e., the slopes of the curves decrease. The pH value for the curves, beginning at the left, are respectively, 4.8, 5.3, 5.8, 6.3, 6.8, 7.3, 7.8, 8.3, and 8.8. With this chart the work of adjusting the acidity of some eighty solutions every two or three days was shortened to the mere checking of a few typical ones.

A few of these cultures were studied in order to determine what effect the precipitation of the iron and the growth of plants in these solutions would have upon the pH of the media. After five days growth under artificial light, the plants were removed from the flasks and pH of the solutions determined. This experiment was repeated later and confirmed. The data obtained was plotted in two ways as shown in Figure V. The ordinates represent the differences between the original and final pH values and are the same for both sets of curves. In the first set the change in pH is plotted against the original pH of the solution, the three curves representing the concentrations of iron used. In the second set of curves the change in pH is plotted against the concentration of iron expressed in mgms./liter. The lowest curve represents the highest original pH.

In all these cultures the pH has increased during the five-day period. The magnitude of the increase, however,

is different for each concentration of iron and each original pH. For all concentrations of iron the greatest rise in pH was from an original value of 4.8 and the least from an original value of 7.3. For any given pH the greatest increase was in a solution containing 0.5 mgms. of iron per liter. Ferric citrate cultures, therefore, cannot be depended upon to maintain a definite pH unless the original value is very close to the neutral point. This effect was not investigated in the more alkaline solutions.

TABLE NO. IV

BUFFERING POWER OF FERRIC CITRATE

(cc of KOH per 125 cc of nutrient solution required to bring each concentration of ferric citrate to the pH stated)

Note: For solutions requiring more than 50 cc of 0.005 N KOH, 0.2 N KOH was used.

Series Number	Conc. of Ferric Citrate mgms./L.	pH:	305	306	307	308	309	310	311	312	313
			4.8	5.3	5.8	6.3	6.8	7.3	7.8	8.3	8.8
A	0.5		2.1	3.5	6.3	10.5	17.7	26.3	---	---	---
B	1.0		2.3	3.8	6.5	10.8	18.0	26.7	---	---	---
C	2.0		2.7	4.4	7.1	11.4	18.6	27.6	---	---	---
D	4.0		3.5	5.5	8.2	12.6	19.8	29.3	---	---	---
E	8.0		5.1	7.6	10.5	15.0	22.3	32.9	50.0	90.8	165.0
F	16.0		8.3	12.0	15.0	19.9	27.3	39.9	58.5	100.3	176.8
G	32.0		14.8	19.4	23.5	29.8	39.1	54.0	76.0	120.0	200.0
H	48.0		21.3	27.1	32.0	39.7	50.8	68.0	91.6	139.2	233.0
I	64.0		27.9	34.7	40.5	49.6	64.0	82.0	108.0	158.4	246.2
J	80.0		34.4	42.4	49.0	60.0	74.0	96.4	124.0	177.6	269.5

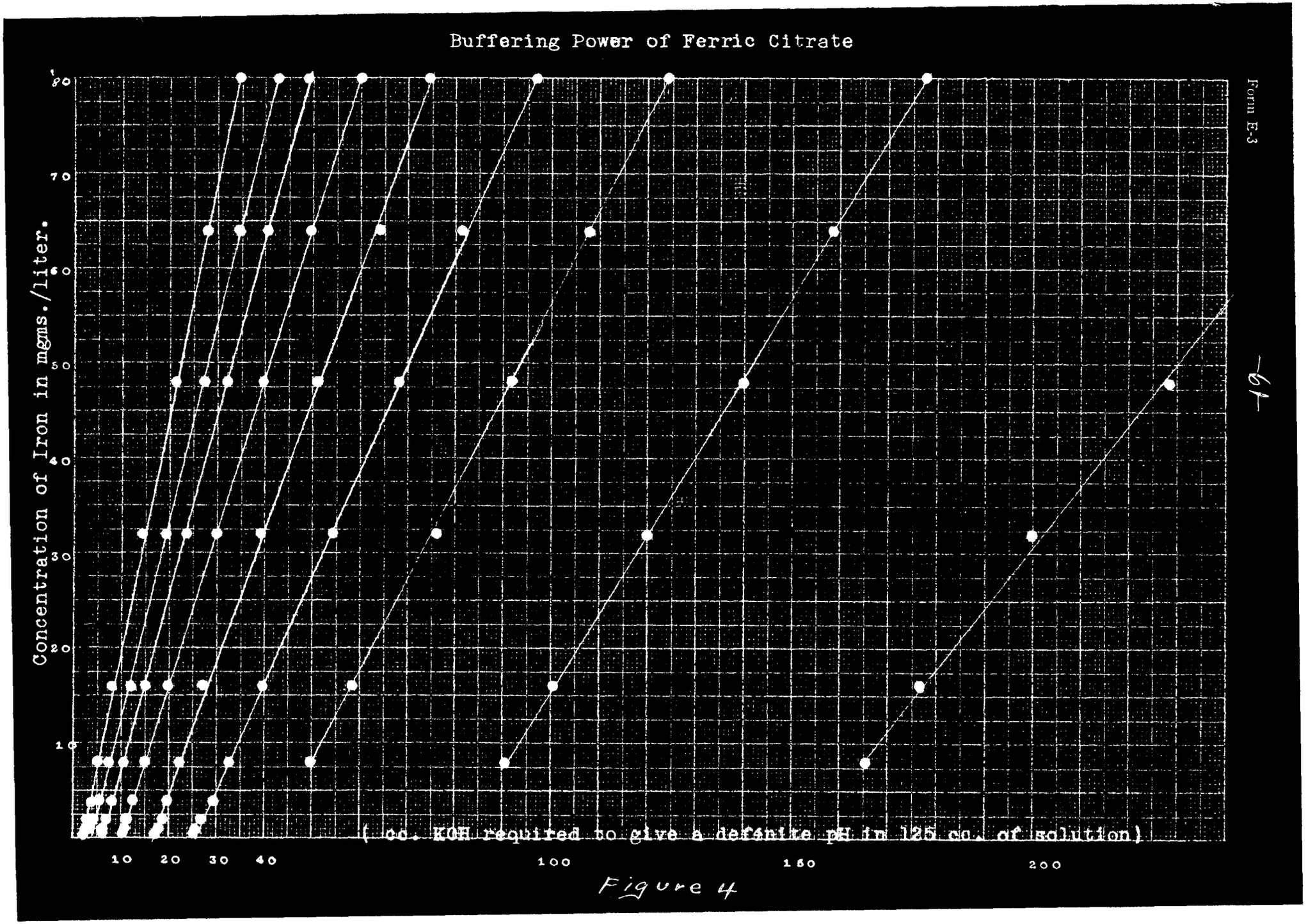

Buffering Power of Ferric Citrate
Concentration of Iron in mgms./liter.
80
70
60
50
40
30
20
10
(cc. KOH required to give a definite pH in 125 cc. of solution)
10
20
30
40
100
150
200

Figure 4

Change in pH after 5 days growth of plants.

Figure 5

Change in pH of culture solutions containing Organic Iron

Form E-3

ORGANIC IRON

II. THE EFFECT OF ORGANIC IRON UPON THE GROWTH OF LEMNA IN CULTURE SOLUTIONS.

Investigations on the growth of Lemna major in solutions containing organic iron were carried out in the same manner as the experiments described for inorganic iron. The data are presented in Tables V and VI in the manner previously described. Growth curves are given in Figures VI, VII and VIII for typical cultures. Table VI describes the appearance and behavior of Lemna grown in solutions representing the entire range of concentrations of iron and of hydrogen ion used in this study. A number of other experiments were carried out later. Unless otherwise described, however, the general appearance and behavior of Lemna in these cultures may be assumed to be similar to those of corresponding iron concentration and pH described in Table VI. No table describing the plants in each individual culture is given for these later experiments.

TABLE NO. V

GROWTH OF LEMNA IN ORGANIC CULTURE SOLUTIONS

Number of Culture	Source of Iron	Conc. of Iron Mgms./L.	pH	Conc. of Mn mgms./L.	Treatment of Cultures (n-s indicates non-sterile) (CLTA indicates Constant Light and Temperature Apparatus)	Growth K x 100
305A-	Ferric Citrate	0.5	4.8	1.0	Non-sterile cultures grown in CLTA	5.1
305B	"	1.0	"	"	" " " " " "	5.2
305C	"	2.0	"	"	" " " " " "	5.5
305D	"	4.0	"	"	" " " " " "	6.0
305E	"	8.0	"	"	" " " " " "	6.3
305F	"	16.0	"	"	" " " " " "	7.5
305G	"	32.0	"	"	" " " " " "	8.3
305H	"	48.0	"	"	" " " " " "	8.6
305I	"	64.0	"	"	" " " " " "	8.8
305J	"	80.0	"	"	" " " " " "	9.2
306A	"	0.5	5.3	"	" " " " " "	4.9
306B	"	1.0	"	"	" " " " " "	5.3
306C	"	2.0	"	"	" " " " " "	5.8
306D	"	4.0	"	"	" " " " " "	6.2
306E	"	8.0	"	"	" " " " " "	6.5
306F	"	16.0	"	"	" " " " " "	8.0
306G	"	32.0	"	"	" " " " " "	8.6
306H	"	48.0	"	"	" " " " " "	9.0
306I	"	64.0	"	"	" " " " " "	9.3
306J	"	80.0	"	"	" " " " " "	9.6
307A	"	0.5	5.8	"	" " " " " "	3.9
307B	"	1.0	"	"	" " " " " "	5.1
307C	"	2.0	"	"	" " " " " "	6.0
307D	"	4.0	"	"	" " " " " "	6.5
307E	"	8.0	"	"	" " " " " "	6.8
307F	"	16.0	"	"	" " " " " "	8.3

TABLE NO. V (continued)

GROWTH OF LEMNA IN ORGANIC CULTURE SOLUTIONS

Number of Culture	Source of Iron	Conc. of Iron Mgms./L.	pH	Conc. of Mn mgms./L.	Treatment of Cultures (n-s indicates non-sterile) (CLTA indicates Constant Light and Temperature Apparatus)	Growth K x 100
307G	Ferric Citrate	32.0	5.8	1.0	Non-sterile cultures grown in CLTA	9.0
307H	"	48.0	"	"	" " " " " "	9.4
307I	"	64.0	"	"	" " " " " "	10.0
307J	"	80.0	"	"	" " " " " "	10.2
308A	"	0.5	6.3	"	" " " " " "	3.1
308B	"	1.0	"	"	" " " " " "	4.6
308C	"	2.0	"	"	" " " " " "	5.5
308D	"	4.0	"	"	" " " " " "	7.0
308E	"	8.0	"	"	" " " " " "	7.5
308F	"	16.0	"	"	" " " " " "	8.5
308G	"	32.0	"	"	" " " " " "	9.5
308H	"	48.0	"	"	" " " " " "	9.9
308I	"	64.0	"	"	" " " " " "	10.3
308J	"	80.0	"	"	" " " " " "	10.5
309A	"	0.5	6.8	"	" " " " " "	2.8
309B	"	1.0	"	"	" " " " " "	3.9
309C	"	2.0	"	"	" " " " " "	4.6
309D	"	4.0	"	"	" " " " " "	6.0
309E	"	8.0	"	"	" " " " " "	8.3
309F	"	16.0	"	"	" " " " " "	9.5
309G	"	32.0	"	"	" " " " " "	10.0
309H	"	48.0	"	"	" " " " " "	10.3
309I	"	64.0	"	"	" " " " " "	10.5
309J	"	80.0	"	"	" " " " " "	10.8

TABLE NO. V (continued)

GROWTH OF LEMNA IN ORGANIC CULTURE SOLUTIONS

Number of Culture	Source of Iron	Conc. of: Iron Mgms./L.	pH	Conc. of: Mn Mgms./L.	Treatment of Cultures (n-s indicates non-sterile) (CLTA indicates Constant Light and Temperature Apparatus)	Growth K x 100
310A	Ferric Citrate	0.5	7.3	1.0	Non-sterile; grown in CLTA	8.5
310B	"	1.0	"	"	" " " " "	3.3
310C	"	2.0	"	"	" " " " "	3.9
310D	"	4.0	"	"	" " " " "	5.0
310E	"	8.0	"	"	" " " " "	8.0
310F	"	16.0	"	"	" " " " "	9.8
310G	"	32.0	"	"	" " " " "	10.4
310H	"	48.0	"	"	" " " " "	10.6
310I	"	64.0	"	"	" " " " "	10.9
310J	"	80.0	"	"	" " " " "	11.2

(Note: The first four concentrations of Ferric Citrate are discontined.)

311E	Ferric Citrate	8.0	7.8	1.0	Non-sterile; grown in CLTA	7.9
311F	"	16.0	"	"	" " " " "	9.4
311G	"	32.0	"	"	" " " " "	10.9
311H	"	48.0	"	"	" " " " "	11.2
311I	"	64.0	"	"	" " " " "	11.6
311J	"	80.0	"	"	" " " " "	11.7
312E	"	8.0	8.3	"	" " " " "	7.8
312F	"	16.0	"	"	" " " " "	8.8
312G	"	32.0	"	"	" " " " "	10.5
312H	"	48.0	"	"	" " " " "	10.3
312I	"	64.0	"	"	" " " " "	9.9
312J	"	80.0	"	"	" " " " "	9.5

TABLE NO. V (continued)

GROWTH OF LEMNA IN ORGANIC CULTURE SOLUTIONS

Number of Culture	Source of Iron	Conc. of Iron Mgms./L.	pH	Conc. of Mn Mgms./L.	Treatment of Culture (n-s indicates non-sterile) (CLTA indicates Constant Light and Temperature Apparatus)	Growth K x 100
313E	Ferric Citrate	8.0	8.8	1.0	Non-sterile; grown in CLTA	7.5
313F	"	16.0	"	"	" " " " "	8.5
313G	"	32.0	"	"	" " " " "	9.5
313H	"	48.0	"	"	" " " " "	8.7
313I	"	64.0	"	"	" " " " "	8.0
313J	"	80.0	"	"	" " " " "	7.7
569A	"	8.0	6.3	0.2	" " " " "	10.3
569B	"	8.0	7.3	0.2	" " " " "	8.8
419A	"	0.5	4.8	0.0	" " " " "	8.2
419B	"	1.0	5.3	"	" " " " "	8.1
419C	"	2.0	5.8	"	" " " " "	8.5
419D	"	4.0	6.3	"	" " " " "	8.5
419E	"	8.0	6.8	"	" " " " "	10.7
419F	"	16.0	7.3	"	" " " " "	11.2
419G	"	32.0	7.8	"	" " " " "	11.7
419H	"	48.0	7.8	"	" " " " "	12.3
419I	"	64.0	7.8	"	" " " " "	12.1
419J	"	80.0	7.8	"	" " " " "	13.3
305A	"	0.5	4.8	1.0	" " " " "	7.6
306B	"	1.0	5.3	"	" " " " "	7.2
307C	"	2.0	5.8	"	" " " " "	8.2
308D	"	4.0	6.3	"	" " " " "	8.6
309E	"	8.0	6.8	"	" " " " "	9.7
310F	"	16.0	7.3	"	" " " " "	10.3

TABLE NO. V (continued)

GROWTH OF LEMNA IN ORGANIC CULTURE SOLUTIONS

Number of Culture	Source of Iron	Conc. of Iron Mgms./L.	pH	Conc. of Mn Mgms./L.	Treatment of Culture (n-s indicates non-sterile) (CLTA indicates Constant Light and Temperature Apparatus)	Growth K x 100
311G	Ferric Citrate	32.0	7.8	1.0	Non-sterile; grown in CLTA	10.8
311H	"	48.0	7.8	"	" " " " "	11.8
311I	"	64.0	7.8	"	" " " " "	12.0
311J	"	80.0	7.8	"	" " " " "	12.0
420	"	32.0	7.8	1.0	Solution sterilized; plants n-s; CLTA	10.0
421	"	32.0	"	0.0	" " " " "	10.4
518	"	64.0	"	1.0	" " " " "	10.0
519	"	64.0	"	0.0	" " " " "	8.8
520	"	32.0	"	1.0	" " " " "	8.7
521	"	32.0	"	0.0	" " " " "	9.6
522	"	32.0	"	0.2	" " " " "	8.6
523	"	8.0	6.8	1.0	" " " " "	5.8
524	"	8.0	6.8	0.0	" " " " "	8.1
525	"	0.5	4.8	1.0	" " " " "	6.1
526	"	0.5	4.8	0.0	" " " " "	5.7
556E	"	32.0	7.8	1.0	" " " sterilized "	8.8
556F	"	32.0	"	0.0	" " " " "	8.0
741A	"	"	"	4.0	Completely sterile cultures in CLTA	8.7
741B	"	"	"	2.0	" " " " "	10.0
741C	"	"	"	0.5	" " " " "	9.8
741D	"	"	"	0.2	" " " " "	8.2
741E	"	"	"	0.02	" " " " "	10.6
741F	"	"	"	0.00	" " " " "	8.3

TABLE NO. V (Continued)

GROWTH OF LEMNA IN ORGANIC CULTURE SOLUTIONS

Number of Culture	Source of Iron	Conc. of Iron Mgms./L.	pH	Conc. of Mn Mgms./L.	Treatment of Culture (n-s indicates non-sterile) (CLTA indicates Constant Light and Temperature Apparatus)	Growth K x 100
776A	Ferric Citrate	0.5	4.8	0.00	Completely sterile cultures in CLTA	6.2
776B	"	"	"	4.0	" " " " "	7.7
776C	"	"	"	2.0	" " " " "	8.2
776D	"	"	"	0.5	" " " " "	8.7
776E	"	"	"	0.2	" " " " "	6.2
776F	"	"	"	0.02	" " " " "	7.6
846	"	32.0	7.8	0.02	9 cc $CaH_4(PO_4)_2$ added; sterile; CLTA	9.2
346A	"	0.5	4.8	0.0	Knop's solution; non-sterile grown in (CLTA	7.3
346B	"	5.0	4.8	"	" " " " "	5.7
346C	"	16.0	4.8	"	" " " " "	7.4
347A	"	0.5	6.3	"	" " " " "	5.0
347B	"	5.0	6.3	"	" " " " "	6.0
347C	"	16.0	6.3	"	" " " " "	11.2
348A	"	0.5	7.6	"	" " " " "	3.5
348B	"	5.0	7.6	"	" " " " "	4.5
348C	"	16.0	7.6	"	" " " " "	10.0

TABLE NO. VI

DESCRIPTION OF THE APPEARANCE AND BEHAVIOR OF LEMNA IN ORGANIC CULTURE SOLUTIONS

Number of Culture	Source of Light	Description
305A	CLTA	medium to large, shiny green, round, thick, short roots, deep red tinge.
305B	"	" " " " " " " " " " " "
305C	"	" " " " " " " " " " " "
305D	"	" " " " " " " " " " " "
305E	"	large, green but not glossy, longer roots, slight reddish tinge.
305F	"	very large; normal green color; long roots, reddish tinge absent.
305G	"	large, green; tendency to lose roots.
305H	"	medium to small with brown spots, less chlorophyll, lost roots.
305I	"	" " " " " " yellow-green; oily scum.
305J	"	small, yellow, brown spots more abundant; more oily scum; lost roots.
306A	"	medium size; yellow-green; lost roots; reddish tinge.
306B	"	medium to large; green; reddish tinge, short roots.
306C	"	" " " " " " " lost roots.
306D	"	" " " yellow to green; lost roots; algae noticeable.
306E	"	large; medium green; lost roots; algae present.
306F	"	very large and green; lost roots; roots dark; algae present.
306G	"	medium size; yellow-green; lost roots; algae present.
306H	"	medium to small; yellow-green; brown spots; lost roots; algae abundant.
306I	"	small; yellow-green; oily scum; lost roots; algae not abundant.
306J	"	" " " " " " " " present.
307A	"	small; yellow; lost roots; slight reddish tinge.
307B	"	" " " " " " "
307C	"	medium to large; green; glossy; reddish tinge; lost roots.
307D	"	medium to large; medium green; lost roots; algae present.
307E	"	large; medium green; lost roots; reddish tinge; algae present.
307F	"	large; green; lost roots; algae present.

TABLE NO. VI (continued)

DESCRIPTION OF THE APPEARANCE AND BEHAVIOR OF LEMNA IN ORGANIC CULTURE SOLUTIONS

Number of Culture	Source of Light	Description
307G	CLTA	large; green; lost roots; algae present.
307H	"	medium to small; pale green; oily scum; lost roots; algae abundant.
307I	"	" " " " " " " " few algae.
307J	"	" " " glossy green; oily scum; lost roots; no algae.
308A	"	small and yellow; no growth.
308B	"	" " " lost roots; slight reddish tinge.
308C	"	" " " " " " "
308D	"	medium to large; glossy green; reddish tinge; lost roots.
308E	"	" " " green; slight reddish tinge; lost roots; algae present.
308F	"	very large and green with few algae and slight loss of roots.
308G	"	large; green; lost roots; algae present.
308H	"	medium to large; green; lost roots; algae abundant.
308I	"	" " " " " " " " oily scum.
308J	"	" " " " " " few algae " "
309A	"	small; very yellow; dead within a few days.
309B	"	" " " lost roots, very light reddish tinge.
309C	"	" " " " " " " " "
309D	"	medium; yellow; lost roots; reddish tinge.
309E	"	very large; medium green; lost roots; reddish tinge; few algae.
309F	"	very large; green; slight loss of roots; algae present.
309G	"	medium to large; green.
309H	"	medium; medium green; lost roots.
309I	"	" " " " "
309J	"	medium to large; yellow-green; lost roots; algae present.

TABLE NO VI (continued)

DESCRIPTION OF THE APPEARANCE AND BEHAVIOR OF LEMNA IN ORGANIC CULTURE SOLUTIONS

Number of Culture	Source of Light	Description
310A	CLTA	died very quickly.
310B	"	were dying at end of experiment.
310C	"	" " " " " "
310D	"	" " " " " "
310E	"	medium size; yellow-green; lost roots; slight reddish tinge.
310F	"	very large and green; lost roots in some cultures.
310G	"	large; green; slight loss of roots.
310H	"	medium to large; medium green; lost roots; slight oily scum.
310I	"	large; medium green; lost roots; algae present.
310J	"	medium; green " " " "
311E	"	medium size; yellow-green; lost roots; algae abundant.
311F	"	" " " " " " " "
311G	"	large; green; lost roots in cultures with algae present.
311H	"	medium; green.
311I	"	large; green; lost roots in cultures containing algae.
311J	"	" " " " " " " "
312E	"	small; yellow; lost roots.
312F	"	medium; yellow-green; lost roots.
312G	"	large; medium green; lost roots; algae present.
312H	"	medium; pale green; lost roots; brown spots.
312I	"	" " " " " algae present.
312J	"	" " " brown roots.

TABLE NO. VI (continued)

DESCRIPTION OF THE APPEARANCE AND BEHAVIOR OF LEMNA IN ORGANIC CULTURE SOLUTIONS

Number of Culture	Source of Light	Description
313E	CLTA	small; yellow-green; lost roots; brown spots.
313F	"	medium; " " " " algae present.
313G	"	medium size; pale green; lost roots; brown spots.
313H	"	small; yellow-green; lost roots; brown spots.
313I	"	small; pale green; brown roots; brown spots abundant.
313J	"	" " " " " " " "

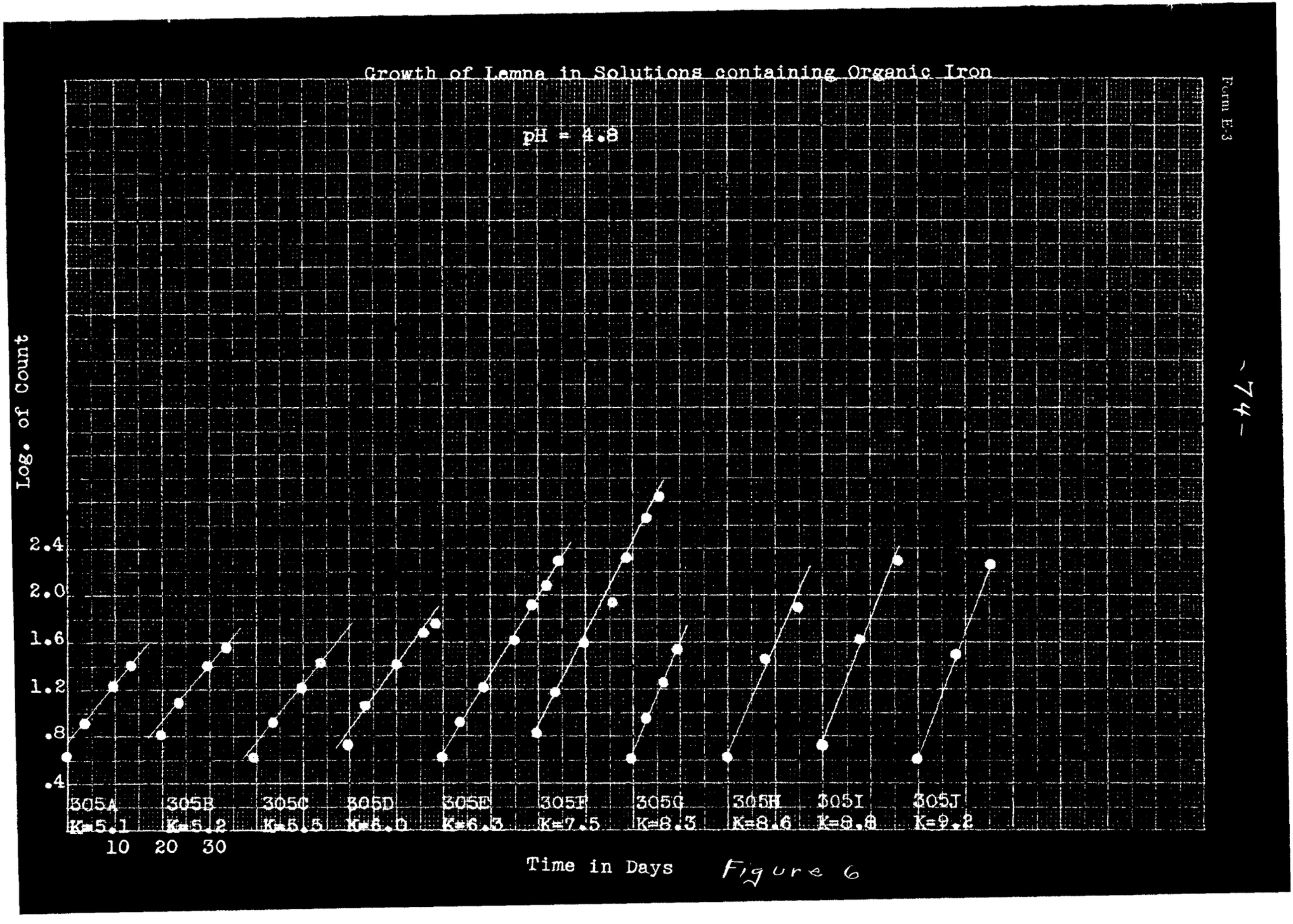


Figure 6

Form E-3

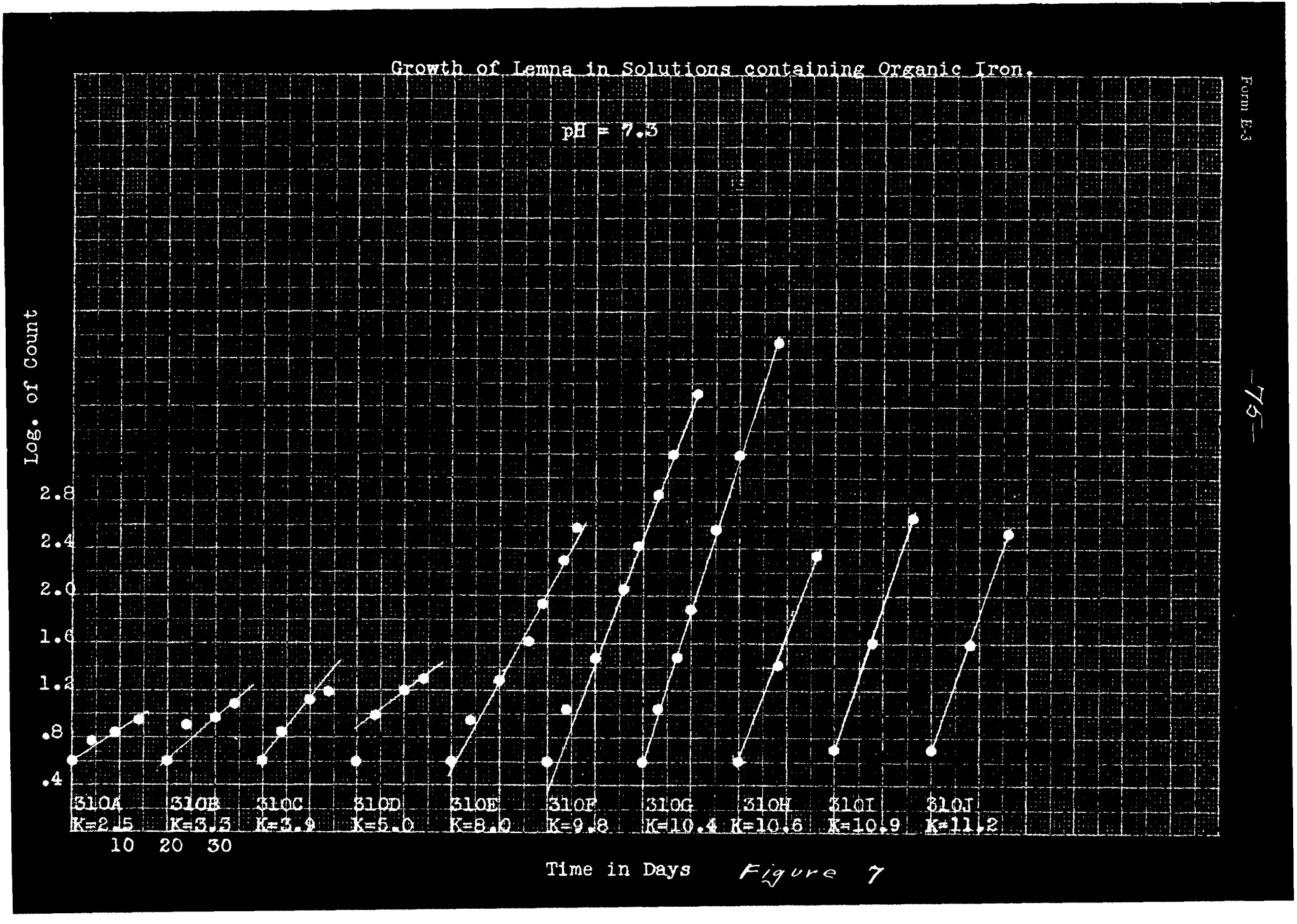

Growth of Lemna in Solutions containing Organic Iron.
pH = 7.3
Log. of Count
2.8
2.4
2.0
1.6
1.2
.8
.4
310A K=2.5
310B K=3.3
310C K=3.9
310D K=5.0
310E K=8.0
310F K=9.8
310G K=10.4
310H K=10.6
310I K=10.9
310J K=11.2
10
20
30
Time in Days

Figure 7

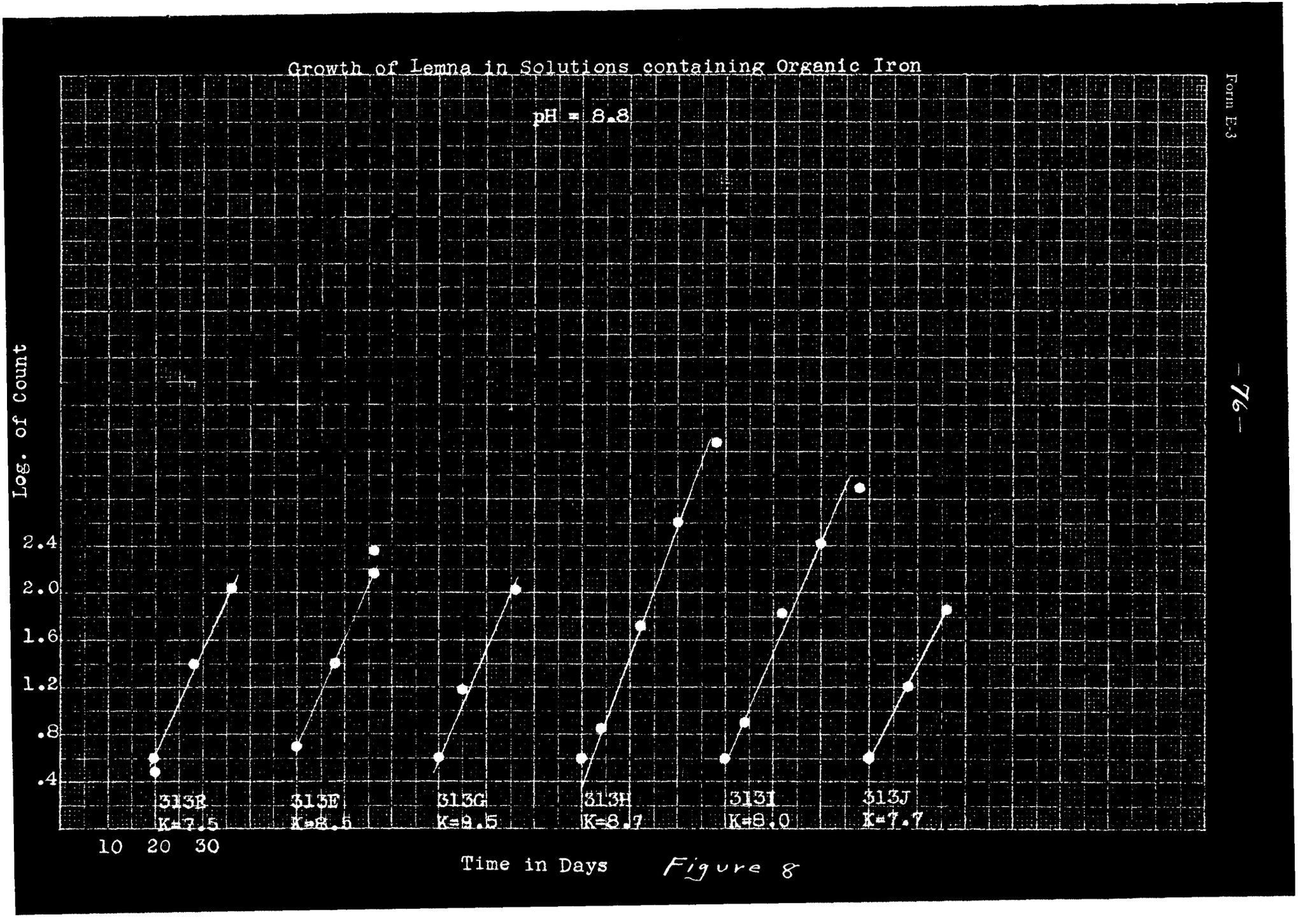

Growth of Lemna in Solutions containing Organic Iron
pH = 8.8
Log. of Count
2.4
2.0
1.6
1.2
.8
.4
10
20
30
Time in Days
313R
K=7.5
313E
K=8.6
313G
K=9.5
313H
K=8.7
313I
K=8.0
313J
K=7.7

Figure 8

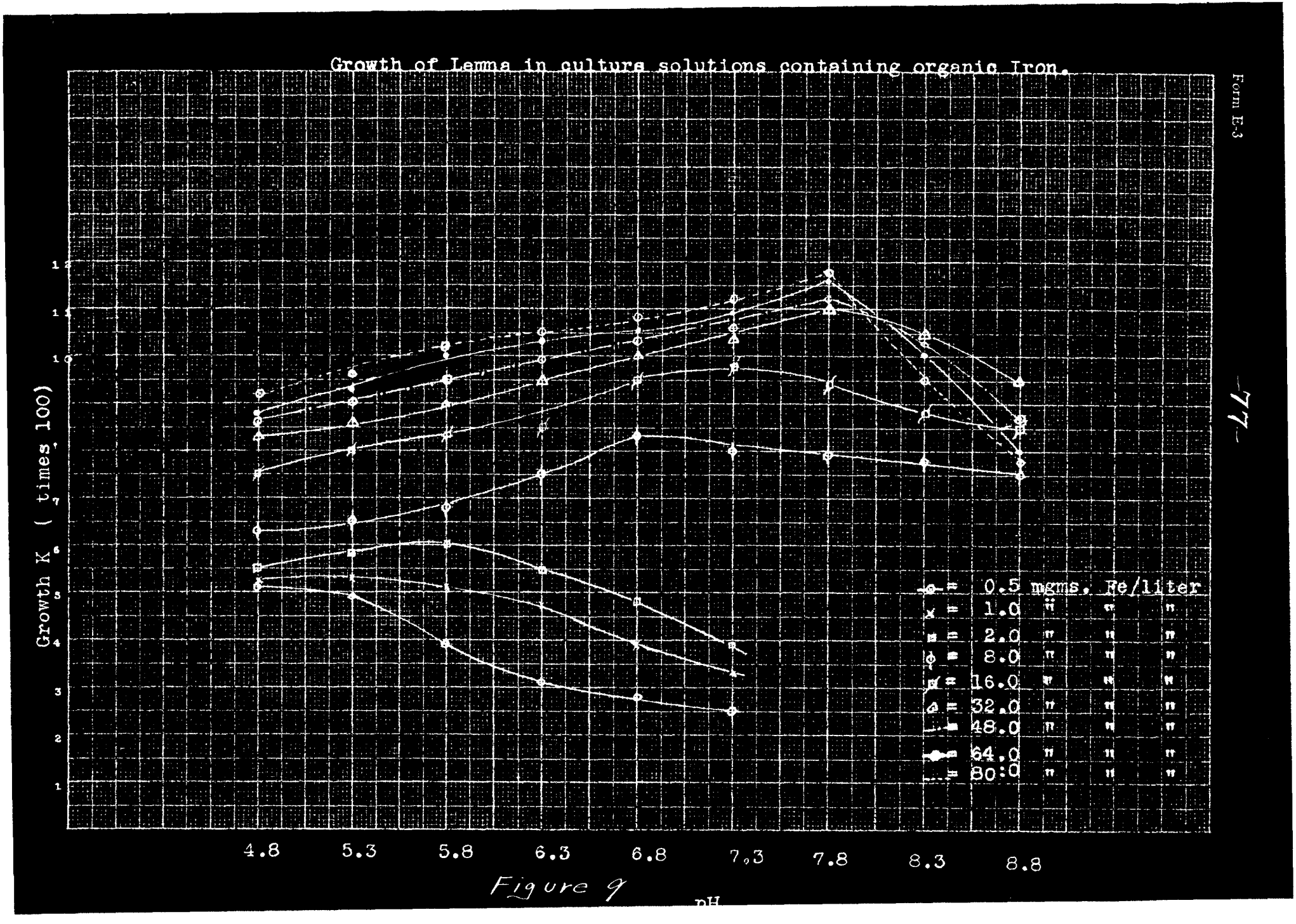

Growth of Lemna in culture solutions containing organic Iron.
Growth K (times 100)
pH
4.8
5.3
5.8
6.3
6.8
7.3
7.8
8.3
8.8
0.5 mgms. Fe/liter
1.0 " " "
2.0 " " "
8.0 " " "
16.0 " " "
32.0 " " "
48.0 " " "
64.0 " " "
80.0 " " "

Figure 9

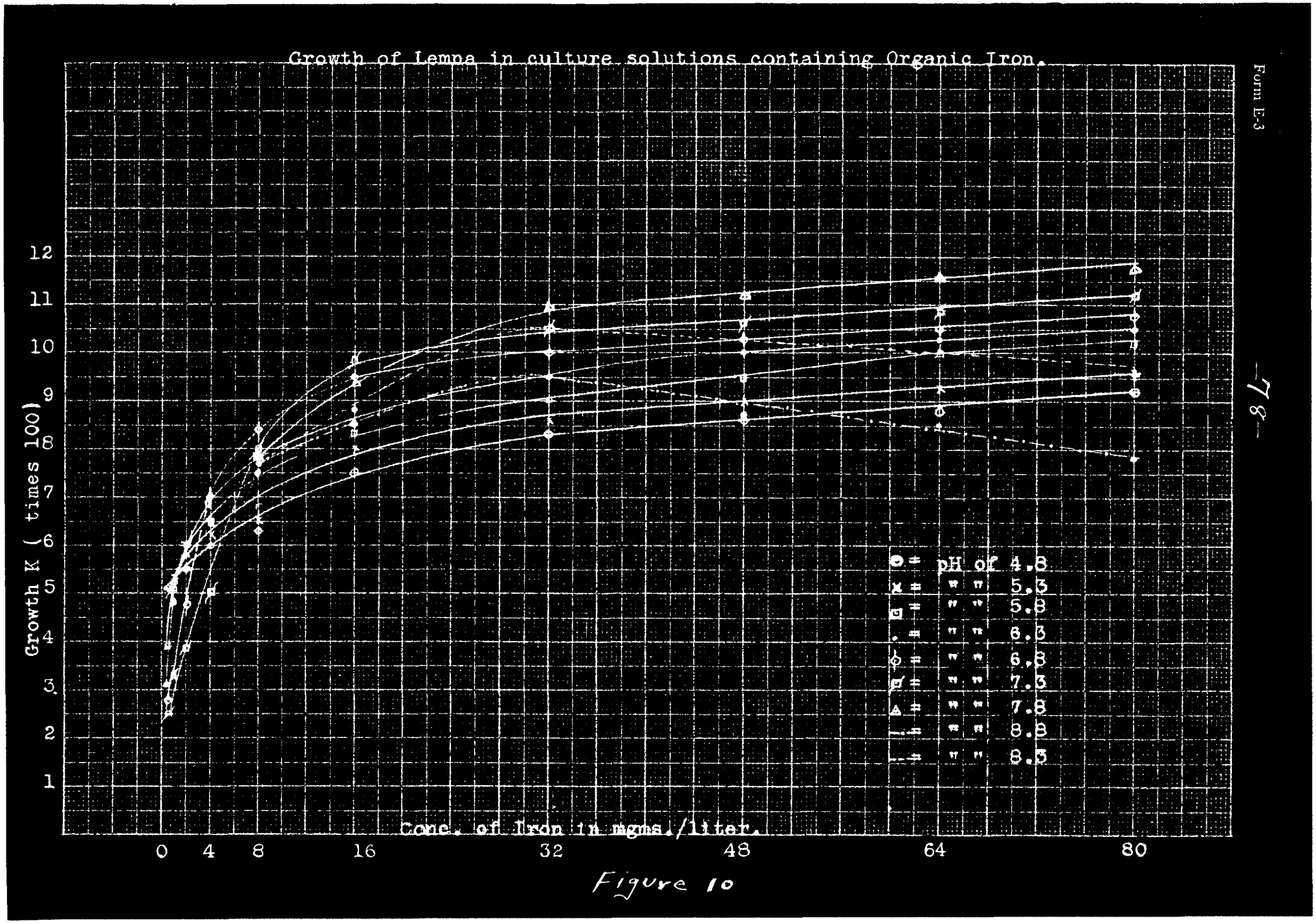

Growth of Lemna in culture solutions containing Organic Iron.
Growth K (times 100)
12
11
10
9
8
7
6
5
4
3
2
1
Conc. of Iron in mgms./liter.
0
4
8
16
32
48
64
80
○ = pH of 4.8
× = " " 5.3
□ = " " 5.8
· = " " 6.3
◇ = " " 6.8
▽ = " " 7.3
△ = " " 7.8
_ = " " 8.3
_ = " " 8.3

Figure 10

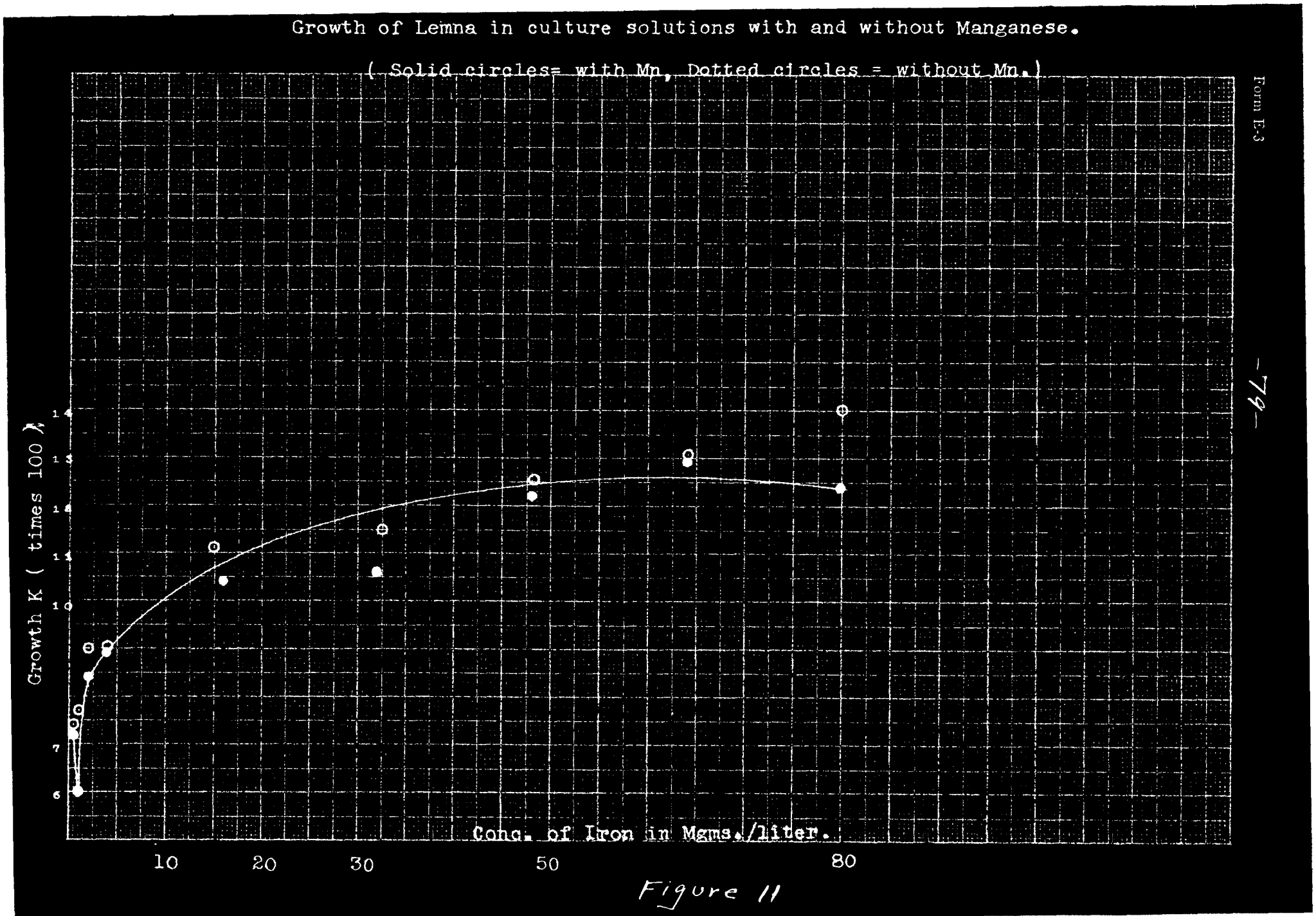

Growth of Lemna in culture solutions with and without Manganese.
(Solid circles= with Mn, Dotted circles = without Mn.)
Growth K (times 100)
14
13
12
11
10
7
6
Conc. of Iron in Mgms./liter.
10
20
30
50
80

Figure 11

DISCUSSION OF EXPERIMENTAL RESULTS

The cultures 305A-J to 313E-J cover a range of hydrogen ion concentrations from 4.8 to 8.8 and a range of iron concentrations from 0.5 to 80.0 milligrams of iron per liter. It should be kept in mind that these figures represent the values obtained for the fresh solutions. The changes which take place in the solutions during the time (two or three days) the plants are growing in them, are described under preliminary experiments (pages 53 to 59, and Figure V). The description of the plants grown in these series of cultures is given in Table VI. Plants refused to grow in cultures having a pH above 7.3 with iron concentrations below 8.0 mgms. per liter. Growth in these concentrations of iron, therefore, were not investigated above pH 7.3.

The relationships of pH and iron concentration to the reproduction of Lemna are brought out clearly in the graphical studies of the growth rates given in Figures IX and X (pages 77, 78). For each concentration of organic iron supplied to the nutrient solution there is an optimum pH range. (See Figure IX). Up to a pH of 7.8 this optimum is different for each concentration of iron used. A pH of 7.8 seems, however, to be the upper limit for any concentration of iron since the growth rate is depressed in all cultures having a pH higher than 7.8. The lowest concentration of iron (0.5 mgms./liter)

has an optimum pH of 4.8. This is the value which Clark (1926) found best for Lemna grown in his solution containing $FeCl_3$ at the same concentration. The plants (305A) in the organic culture were larger and greener than those in the inorganic (323). For all pH values the growth of Lemna increased rapidly with increasing concentrations of iron up to 8 to 16 mgms./liter, then increased slowly up to 80 mgms./liter. Above pH 7.8 the upper three concentrations of iron depressed the growth rate. The optimum pH rises with each increase in the concentration of ferric citrate up to a pH of 7.8. Beyond 7.8 a concentration of 32 mgms./liter produced more rapid growth than the higher concentrations. In fact successively increasing the concentrations of iron from 32 to 80 mgms./liter in solutions more alkaline than pH 7.8, caused a corresponding decrease in the growth rate. This is brought out clearly in Figure X. For all pH values selected, up to 7.8, the highest rate of reproduction is obtained in those solutions having the highest concentration of iron. Selecting the optimum pH for each concentration of iron the following relationships are noted: 0.5 mgms./liter pH 4.8, 1.0-5.3, 2.0-5.8, 4.0-63, 8.0-6.8, 16-7.3, 32-7.8, 48-7.8, 64-7.8, 80-7.8. Thus, while the upper limit of hydrogen ion concentration has been reached, further increases in the concentration of iron, at the optimum pH values, should produce higher rates of reproduction of Lemna.

An examination of the appearance and behavior of the plants, described in Table VI, shows that the points selected (on the basis of growth rate) as optimum for the various concentrations of iron seem to be also optimum for size, depth of color and general appearance of the plants. The plants were healthy in all used concentrations of hydrogen ion below the optimum value for any particular amount of iron, except for the concentrations 48, 64, and 80 mgms./liter. These were toxic in the more acid solutions, producing stunted plants which were yellow, brown spotted and unable to retain roots. It was also noted that algae would not grow in these solutions. The plants became unhealthy, small, chlorotic and unable to retain roots, as the pH was increased above the optimum. In many of the cultures it was difficult to prevent contamination with algae even by changing the solutions every two or three days. Due to the large number of experiments, effects caused by algae were easily detected and the unhealthy plants replaced by healthy ones from stock cultures.

Since all the above cultures contained 1 p.p.m. of manganese, a set of experiments were carried out to note the effect of omitting this element. Solutions 419A-J (page 67) representing a range of 0.5 to 80.0 mgms. of iron per liter were adjusted to the optimum pH for each concentration of iron; the values having been determined from the previous experiment. The manganese was omitted in these solutions but a similar

series (305A-311J, pages 67, 68) containing 1 p.p.m. of manganese was used as a check. That the growth rates for similar cultures do not check with those obtained in the first experiment is due to a change in the source of light and time of illumination.

The growth rates, while not uniform, show clearly in the graphical analyses presented in Figure XI, that manganese depresses the rate of growth in all cultures. The appearance of the plants was also better in the solutions containing no manganese. This conclusion did not apply to the algae present in the cultures; these grew well in the solutions containing manganese but poorly when this element was omitted. This tends to confirm the work of Hopkins (1930) with algae; he found that manganese was essential for the growth of Chlorella.

Experiments 420 and 421 (Conc. of iron-32.0 p.p.m., pH 7.8) were sterilized each time before receiving the (non-sterile) plants. No algae developed in these cultures and the plants were large and green. The solution containing manganese again produced slower growth of Lemna. Cultures containing other concentrations of iron at their optimum values of pH were treated in the same manner (See 518-26). Lowering of the growth rate due to the presence of manganese is noticed in the cultures containing 32 (520-522) and 8

(523-524) milligrams of iron per liter, but not in concentrations of 64 (518-519) and 0.5 (525, 526) mgms./liter. (First number refers to solutions containing manganese.) In these cultures the appearance of the plants was also better in the solutions containing no manganese.

It was hoped that complete sterilization might give more consistent results than obtained above with partial sterilization. Preliminary experiments (556E, 556F) indicated a higher growth rate with manganese in the sterile cultures. The plants in both solutions were not as large as those grown under non-sterile conditions but were green and otherwise healthy in appearance. Two series of cultures, in which the manganese content was varied, were studied. Solutions 7141A-F (pH 7.8, iron 32 mgms./liter) and 759A-F (pH 4.8, iron 0.5 mgms./liter) received concentrations of manganese from 0.02 to 4.0 p.p.m., one without manganese being used as a check. The experiment was continued for 24 days and the plants were healthy and green throughout this time. In neither of these series was there a correlation of the manganese concentration with the rate of growth of Lemna. In both series, however, the cultures receiving no manganese grew at a slower rate than any of the other cultures. It appears, therefore, that concentrations of manganese, which depress the growth of Lemna in non-sterile organic cultures, may stimulate growth when the plants and solutions are sterilized. This result contrasts

with the effect of manganese in cultures receiving inorganic iron. In the inorganic solutions sterile plants grew equally well with or without manganese; the higher concentrations, however, were toxic.

An interesting study of the effects of partial and complete sterilization may be made by comparing similar cultures which have been subjected to these treatments. Thus, solutions receiving 32 mgms. of iron per liter (pH 7.8) and no manganese show the following rates of growth: non-sterile, (419A), 11.7, solutions sterilized; plants non-sterile (522) 9.6 and (421) 10.4; and sterile cultures (556F) 8.0 and (741F) 8.3 Concentrations of iron of 0.5 mgms./liter (419A, 526, 305A, 525) and 64 mgms./liter (419I, 519, 311I, 518) show similar depression of growth by partial and complete sterilization. It is noted that these results contrast also with experiments on inorganic iron; in the inorganic media the sterile plants reproduced more rapidly than those grown in non-sterile or partially-sterilized solutions. These results confirm the work of Clark and Roller (1931) who showed that organic matter depresses the growth of sterile cultures of Lemna.

The optimum pH for the growth of Lemna in solutions receiving 0.5 mgms. of iron per liter is 4.8 when either $FeCl_3$ or ferric citrate is used as the source of iron. A comparison of growth rates of non-sterile plants in solutions

containing $FeCl_3$ and ferric citrate respectively, show the following: (323, 305A) 7.0-7.6, (527, 526) 5.0-5.7, (528, 525) 4.0-6.1. In sterile cultures the growth rates produced by increasing amounts of manganese are for $FeCl_3$ and ferric citrate respectively: (759A, 776A) 8.2-6.2, (759D, 776F) 8.8-7.6, (759C, 776E) 8.9-6.2, (749B, 776D) 7.5-8.7, (759E, 776C) 7.0-8.2, and (759F, 776B) 7.9-7.7. In these experiments it is noted that under non-sterile conditions organic iron stimulates the growth of the plants; under sterile conditions faster reproduction is produced by inorganic iron, at the same concentration, except in the solutions containing toxic concentrations of manganese. The higher concentrations of manganese (0.5 to 4.0 p.p.m.) were not toxic in the organic cultures. These results check also the work of Clark and Roller (1931) on the effect of organic matter on the rate of reproduction of Lemna under sterile conditions. It does not appear that the effect of sterilization is upon the availability of the organic iron; the plants were dark green and apparently healthy in both sterile and non-sterile cultures, but were usually smaller in the sterile cultures. There seems to be some effect upon the organic matter which causes a depression of growth in sterile cultures, apart from its relation to the availability of iron.

A review of the above experiments on the effect of organic iron on the growth of Lemna is further evidence for the

advantage gained by using this form of iron for the growth of plants in water cultures.

That increasing the concentration of iron in nutrient solutions may increase production of plants grown in them is shown by Emerson (1929), Gines (1930) and Hopkins (1930). That there is an optimum pH for each concentration of iron added was not reported by these writers but is shown in the work on Lemna. Emerson found he could regulate the chlorophyll content of Chlorella by varying the iron content of a solution containing glucose but could not do so when glucose was absent. He does not state the pH of his medium. Gines found the growth range of plants for a number of inorganic sources of iron to vary between 1 and 133 p.p.m. of iron but control of the pH was not stated. Hopkins grew Chlorella in a solution having a pH of 7.2, and the reason that the pH of his medium did not change is probably due to the fact that the original hydrogen concentration was near the neutral point. That solutions having a pH near 7.0 do not change appreciably on standing is shown in Figure V, and pointed out in the discussion on pages 53 to 59.

The rapid stimulation of the growth of Lemna with increasing amounts of iron in the lower concentrations, and the sharp break to a slower stimulation in the higher concentrations (Figures IX and X) checks closely the work of Hopkins (1930) on Chlorella. The more interesting feature is the relation of pH to growth. That there is an optimum pH for each concentration

of iron, and that the value of the optimum pH increases with increased concentrations of iron up to a pH of 7.8, suggests that when iron is not a limiting factor the metabolism of plant tissues is optimum under neutral or slightly alkaline conditions. It is believed that plants are forced to endure an acid condition in order to obtain sufficient iron for normal growth. That some plants grow well on alkaline soils may be due to a capacity to absorb iron from very low concentrations of iron, or the ability to grow when smaller quantities of iron are present in the tissues of the plant.

The rate of growth of Lemna may be regulated by governing the iron content and pH of the solution. The concentration of chlorophyll has been regulated in Chlorella by governing the iron content of the media. v. Euler, H., and others (1929-1930) have shown that the catalase content of a solution follows closely the content of chlorophyll. Catalase is found by Zeile and Hellstrom (1931) to be an iron porphyrin complex and to be of the same chemical nature in liver extract and in an extract from pumpkin cotyledons. It appears therefore, that the most important chemical processes in plant life, the manufacture of plant food and building material from the CO_2 of the air and from water, and the respiration or oxidation of this material to furnish energy for other metabolic processes, are controlled by iron. That manganese and other elements of its class stimulate the photosynthetic and respiratory processes

has been pointed out, but no element has yet been able to replace iron in life processes and its unique role in life is well established.

The investigations of the effect of manganese in cultures containing organic iron reveal striking contrasts with the results obtained from inorganic iron. In the inorganic cultures manganese did not stimulate growth either under sterile or non-sterile conditions and was toxic in the higher concentrations. Plants in sterile cultures grew much better than in non-sterile, and equally well in the optimum concentrations of manganese or in its absence. With the organic iron, manganese depressed growth of the non-sterile plants, but stimulated growth of the sterile ones, even in concentrations which were toxic in the inorganic solutions. This effect was noted at a pH of 4.8 and a pH of 7.8. In the inorganic cultures manganese was much more toxic in the more alkaline solutions. Consequently organic matter reduces the toxic effect of manganese under sterile conditions. An interesting effect of soils high in organic matter is that in these neither manganese-induced chlorosis nor chlorosis due to lack of manganese occurs. Thus organic matter not only increases the availability of iron but provides sufficient manganese for normal plant growth. Since manganese is not essential for Lemna chlorosis due to a lack of this

element cannot be shown. That organic matter decreases the toxicity of manganese in the more alkaline solutions is shown. In the presence of organic matter manganese may be stimulative to the growth of Lemna. This is in contract to the effect of manganese in inorganic culture solutions as presented in Part I.

ORGANIC IRON

III. PREPARATION OF FERRIC CITRATE.

The U.S.P. ferric citrate is prepared by mixing calculated quantities of the acid and precipitated hydroxide, and evaporating the solution to a point where it will solidify when poured on a porcelain slab. Under these conditions impurities cannot be removed. Some attempts were made therefore to purify ferric citrate for use in culture solutions.

The literature on the composition of ferric citrate and its alkali salts is confusing. Scholz (1908) and Rosenthaler and Siebeck (1908) report the formation of basic iron citrates. The later investigators obtained a compound, by precipitating a hot solution of sodium citrate with $FeCl_3$ or $Fe(NO_3)_3$, which they claimed to be of constant composition. The formula assigned to this compound was $6(FeC_6H_5O_7 \cdot 7Fe(OH)_3 \cdot 9H_2O$. This contains over twice the amount of iron calculated for the normal citrate. Pickering (1913) reports work of early investigators who claimed to have prepared a normal citrate but disagree upon the content of water of hydration. $R_2Fe_2 \cdot 3H_2O$ is reported by one to retain the $3H_2O$ at 100°C but to become anhydrous at 120°C. Another claims the $3H_2O$ is held only under air dry conditions. A salt with $6H_2O$, losing $3H_2O$ at 100°C and all at 150°C, was also reported. Pickering gave as the composition of ferric citrate which is

precipitated by alcohol from a concentrated solution to be $R_3Fe_2.Fe_2O_3.6H_2O$. Analyses showed 24.8-26.6 per cent iron which fell short of the required 29.49 per cent iron. The anhydrous normal salt should contain 22.8 per cent iron.

Belloni (1920) gave as the formula of the alcohol precipitated salt: $(Fe_3(C_6H_5O_7)_2(OH)_2 1/3 C_6H_5O_7.8H_2O$ or $Fe_9(C_6H_5O_7)_6(OH)_6.C_6H_5O_7.24H_2O$ which should analyze 21.29 per cent iron. The two proposed formulas are in discrepancy by 8.2 per cent of iron. Belloni claims 18 H_2O (or 13.74 per cent by weight) lost at 100°C and the other 6 H_2O at 120° prolonged. He claims that when molar amounts of citric acid and iron are mixed 2/9 of the citric acid remains free and suggests $(Fe_3(C_6H_5O_7)_2(OH)_2)OH$ as its base. A chloroplatinate $(Fe_3(C_6H_5O_7)_2(OH)_2)1/2\ PtCl_6.5H_2O$ was obtained. Cowley and Bennett (1928) found that ferric hydroxide dissolved in citric acid reduced the amount of NaOH necessary to neutralize the citric acid up to a ratio of 1.85 gms. Fe_2O_3 to 6.348 gms. citric acid. They claim that the $Fe(OH)_3$ will continue to dissolve up to 5.58 gms. Beyond the ratio 1.85:6.348 Cowley and Bennett claim a colloidal condition exists and that the substance gives a precipitate with saline solutions. Scales, produced by evaporating the neutralized solution containing 1.85 gms. Fe_2O_3, are green; those containing the higher proportions are reddish-black. Pickering reports that the

added $Fe(OH)_3$ may even remove some of that already dissolved to a colloidal state. He found that one mole of iron was actually in solution when 1.23 moles per mole of citric acid had been taken up. When 3 moles of iron had been taken up the soluble iron was found to be 0.744 moles. The ratios of moles of iron to moles of citrate in these various formulas are: Rosenthalier and Siebeck - 13:6, Pickering - 4:3, Belloni - 9:7, and Cowley and Bennett - 5:11. No definite formula can therefore be assigned to ferric citrate.

Attempts were made in this laboratory to obtain a substance the analysis of which might be expected to remain constant. A number of preparations were made using $Fe(OH)_3$ from several different salts and varying the ratio and citric acid. Part of the solution was evaporated to dryness and part precipitated with an equal volume of absolute alcohol. Analyses were made by drying the material in the oven at 50°C, determining the loss of H_2O at 100°, oxidizing the organic matter with HNO_3 and igniting with a dull red heat to constant weight. This method was found to check well with the Zimmerman-Reinhardt method on iron samples of known composition. Of 12 different preparations, using various salts as source of iron hydroxide, the alcohol precipitated compounds gave upon analysis a range of 18.24-25.60 per cent iron with an average of 22.2 per cent. The per cent water lost at 100°C ranged from 4 per cent to 20 per cent. While

the average is close to the percentage of iron in Belloni's compound such wide variation in samples would indicate that no substance of definite composition can be prepared in the manner described by Belloni. The U.S.P. ferric citrate analyzed 20.07 per cent iron; the first alcohol precipitation - 22.5 per cent; and the second alcohol precipitation - 23.07 per cent iron.

Preparation of a ferric citrate of definite composition has not been accomplished. Further work on this substance is needed.

THE STIMULATIVE EFFECT OF ORGANIC MATTER ON THE GROWTH OF LEMNA

In the introductory part of this thesis attention was called to the work of several investigators who have reported stimulation of the growth of green plants by minute quantities of organic extracts added to nutrient solutions. The suggestion that this stimulation was due to the effect of organic matter upon the availability of iron to the plant was investigated. The results show that organic iron compounds are more available to plants than inorganic especially when the plants are growing in alkaline or neutral medium. Indications were found in the sterile cultures that there was some effect upon the plant by organic matter apart from the effect on availability of iron. Experiments were carried out to study further this phenomena.

Attention was drawn earlier in this paper to the relationships existing between catalase and chlorophyll, and the occurrence of iron as an essential constituent of the enzyme. Some preliminary experiments by Raber, O. (1931) indicate that liver extract, which contains catalase and hemoglobin, will prevent the loss of chlorophyll by plants placed in the dark and will bring back the green color to etiolated seedlings.

Experiments which report the effect of liver

extract upon the growth of Lemna major are described in Table VII and VIII. For every culture described in these tables a duplicate was placed in the dark. The plants were allowed to grow, without changing the solutions, for 11 days and the growth rates, color, and general appearance noted. A number selected from those which had been growing in the light were placed in the dark and allowed to remain for 9 days. Another set remained in the dark for two weeks.

In the latter part of this experiment a number of sterile and non-sterile cultures were grown in the light and the solutions changed every two days. The description of these cultures is given at the end of Tables VII and VIII and the growth curves are shown in Figure XII.

TABLE NO. VII

GROWTH OF LEMNA IN CULTURE SOLUTIONS TO WHICH LIVER EXTRACT WAS ADDED

Number of Culture	Source of Iron	Conc. of Iron Mgms./L.	pH	Conc. of Mn Mgms./L.	Treatment of Cultures. (n-s indicates non-sterile) (CLTA indicates grown in constant light and temperature apparatus)	Growth K x 100
556A	Ferric Citrate	32.0	7.8	0.0	1% bacto-peptone agar; sterile; CLTA	8.4
556B	$FeCl_3$	0.5	4.8	0.0	" " " " " "	7.9
556C	"	0.5	4.8	1.0	" " " " " "	8.1
556D	Ferric Citrate	32.0	7.8	1.0	" " " " " "	7.9
588	$FeCl_3$	0.5	4.8	1.0	plus 300 ppm. of liver extract; n-s; CLTA	7.6
590	"	"	"	1.0	" " " " " " sterile "	7.5
592	"	"	"	0.0	" " " " " " n-s "	7.0
594	"	"	"	0.0	" " " " " " sterile "	8.7
596	"	"	4.5	0.2	" " " " " " n-s "	7.8-4.9
598	"	"	4.5	0.2	" " " " " " sterile "	6.9-6.1
605	"	5.0	4.8	1.0	" " " " " " n-s "	6.9
607	"	"	4.8	1.0	" " " " " " sterile "	7.8
609	"	"	4.5	0.2	" " " " " " n-s "	6.3-4.9
611	"	"	4.5	0.2	" " " " " " sterile "	6.0
620	Ferric Citrate	0.5	4.8	1.0	" " " " " " n-s "	4.7-5.2
622	"	"	"	1.0	" " " " " " sterile "	6.0
624	"	"	"	0.0	" " " " " " n-s "	6.1
626	"	"	"	0.0	" " " " " " sterile "	6.3
628	"	32.0	7.8	1.0	" " " " " " n-s "	7.8
630	"	"	"	1.0	" " " " " " sterile "	10.3
632	"	"	"	0.0	" " " " " " n-s "	9.8
634	"	"	"	0.0	" " " " " " sterile "	11.7-3.8
636	"	"	"	0.2	" " " " " " n-3 "	8.6
638	"	"	"	0.2	" " " " " " sterile "	7.8

TABLE NO. VII (continued)

GROWTH OF LEMNA IN CULTURE SOLUTIONS TO WHICH LIVER EXTRACT WAS ADDED

Number of Culture	Source of Iron	Conc. of Iron Mgms./L.	pH	Conc. of Mn Mgms./L.	Treatment of Cultures (n-s indicates non-sterile) (CLTA indicates grown in constant light and temperature apparatus)	Growth K x 100
640	Soil	---	---	---	plus 300 ppm. of liver extract; n-s; CLTA	16.0
642*	"	---	---	---	" " " " " " sterile "	6.0*
644	---	---	---	---	stock - Fe - liver extract n-s "	8.4
646	---	---	---	---	" " " " sterile "	8.6-4.4
652	---	---	---	---	stock - iron - 300 ppm. of liver extract - 150 ppm. of glucose; n-s; CLTA	(1.8-12.0)
654	---	---	---	---	(same as 652 except being sterile)	1.7
656	Ferric Citrate	32.0	7.8	0.2	plus 300 ppm. of liver extract; sterilized by a Berkfeld filter and grown in CLTA	5.3

(Note: Plants in above series were not changed but ones below were changed every two days.)

777	$FeCl_3$	0.5	4.8	0.2	plus 300 ppm. liver extract; n-s; CLTA	13.0
778	"	"	"	"	" " " " " sterile "	8.6
779	"	5.0	"	"	" " " " " n-s "	11.7
780	"	"	"	"	" " " " " sterile "	9.6
781	Ferric Citrate	0.5	"	"	" " " " " n-s "	10.6
782	"	"	"	"	" " " " " sterile "	9.4
783	"	32.0	7.8	"	" " " " " n-s "	11.8
784	"	"	"	"	" " " " " sterile "	11.2
801	Soil	---	---	---	" " " " " n-s "	11.0

* Contaminated

TABLE NO. VII (continued

GROWTH OF LEMNA IN CULTURE SOLUTIONS TO WHICH LIVER EXTRACT WAS ADDED

Number of Culture	Source of Iron	Conc. of Iron Mgms./L.	pH	Conc. of Mn Mgms./L.	Treatment of Cultures. (n-s indicates non-sterile) (CLTA indicates grown in constant light and temperature apparatus)	Growth K x 100
802	---	---	---	---	stock - iron plus 300 ppm. of liver extract;non-sterile and grown in CLTA	9.6
803	---	---	---	---	stock - iron - 300 ppm. liver extract - 100 ppm. glucose;non-sterile; CLTA	8.0-2.3
804	Ferric Citrate	32.0	7.8	0.2	plus 300 ppm.liver extract - 50 ppm. of glucose - 50 ppm.of levulose;n-s;CLTA	12.6
808	"	"	"	"	(same as 804 except being sterile)	14.7
845	---	---	---	---	stock - iron - 300 ppm.liver extract sterilized by Berkfeld filter;in CLTA	7.6

TABLE NO. VIII

DESCRIPTION OF APPEARANCE AND BEHAVIOR OF LEMNA IN CULTURE SOLUTIONS TO WHICH LIVER EXTRACT WAS ADDED

Number of Culture	Source of Light	Description
556A	CLTA	medium size;glossy green color;short roots;thick;round.
556B	"	" " " " " " " " "
556C	"	" " " " " " " " "
556D	"	" " " " " " " " "
588	"	medium size; yellow-green; algae very abundant.
590	"	The young buds come out at right angles to the surface of the water, protruding into the air. At first they are almost colorless. After a few days they acquire green color along the veins and at the same time gradually lower themselves to the surface of the solution. Normal, medium size; green plants are the final result, but each frond separates from the parent frond by an extra long strand.
592	"	medium size; pale green; algae very abundant.
594	"	(same as 590)
596	"	medium size; pale green; algae very abundant.
598	"	(same as 590)
605	"	medium size; yellow-green; algae very abundant.
607	"	(same as 590)
609	"	large; medium green; algae very abundant.
611	"	(same as 590)
620	"	large; pale green; algae very abundant.
622	"	(same as 590)
624	"	medium size; green; algae very abundant.
626	"	(same as 590)
628	"	medium size; lost roots; green; algae very abundant.
630	"	" " dark green.
632	"	" " pale green; algae very abundant.

TABLE NO. VIII (continued)

DESCRIPTION OF APPEARANCE AND BEHAVIOR OF LEMNA IN CULTURE SOLUTIONS TO WHICH LIVER EXTRACT WAS ADDED

Number of Culture	Source of Light	Description
634	CLTA	medium size; green.
636	"	" " " algae very abundant.
638	"	" "
640	"	plants three to four times as large as normal; deep green; heavy roots.
642	"	medium size; green but brownish tips.
644	"	parent fronds still green but new buds very tiny with brown centers.
646	"	medium size; green.
652	"	old plants dying from tips inward; new are very small with brown centers.
654	"	small; pale green.
656	"	medium size; yellow-green; lost roots.
777	"	very large and deep green (similar to 640).
778	"	medium; pale green; lost roots; reddish tinge.
779	"	very large and green.
780	"	medium size; pale green; lost roots; reddish tinge.
781	"	very large and green.
782	"	medium size; pale green; deep reddish tinge.
783	"	medium size; green; some brown spots.
784	"	large; green; reddish tinge.
801	"	very large and green.
802	"	medium size; pale green; brown spots.
803	"	" " " yellow-green; lost roots.
804	"	large and green.
808	"	large and green; reddish tinge.
845	"	small; yellow and green; lost roots.

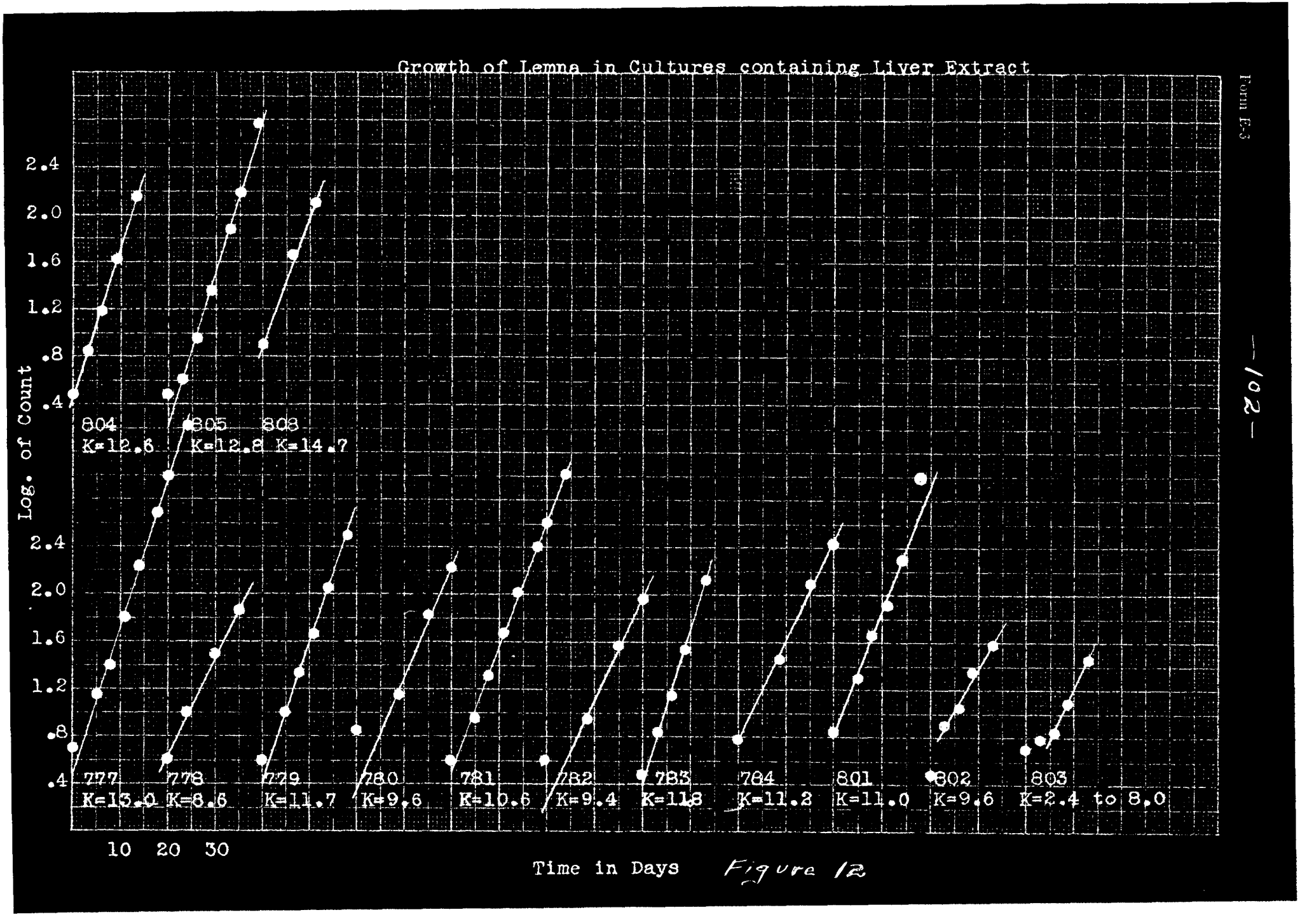

Growth of Lemna in Cultures containing Liver Extract
Log. of Count
2.4
2.0
1.6
1.2
.8
.4
804 K=12.6
805 K=12.8
808 K=14.7
777 K=13.0
778 K=8.6
779 K=11.7
780 K=9.6
781 K=10.6
782 K=9.4
783 K=11.8
784 K=11.2
801 K=11.0
802 K=9.6
803 K=2.4 to 8.0
10 20 30
Time in Days

Figure 12

DISCUSSION OF EXPERIMENTAL RESULTS

In the darkness no reproduction was noticed in any of the cultures, nor could any consistent difference be noted in the chlorophyll content of the plants in solutions receiving liver extract and those in the similar solutions receiving no liver extract. The depth of color was found to be greater in those plants which had received organic iron than in those which had received inorganic. This may have been due, however, to a greater original concentration of chlorophyll in these plants. Two very interesting observations were made. Some cultures from the light which had an abundant growth of algae showed complete disappearance of any traces of algae after a few days in the dark. When these were placed again in the light the development of algae was noted after a short time. Algae then lose their chlorophyll much more quickly than Lemna when placed in the dark. Liver extract did not prevent the loss of chlorophyll.

Two sterile cultures which were grown in a nutrient solution containing 32 p.p.m. of iron as iron citrate and 1 per cent of bacto-peptone agar, showed after 21 days in the dark a deep green color in the plants which were alive although a few fronds were brown and dead. Similar solutions containing liver extract contained plants which were a pale green color. It appears, therefore, that sterile bacto-peptone

agar may contain a substance which prevents the loss of chlorophyll from plants grown in the dark better than liver extract which has been sterilized.

Only the sterile cultures which were grown in the light have any significance in the first set of experiments. The non-sterile cultures were heavily contaminated with algae and in many of the cultures the plants were dead. In nearly all cases the non-sterile cultures showed a lower growth rate than the sterile ones.

In the sterile cultures which contained a low concentration of iron (0.5 p.p.m.) either as $FeCl_3$ or ferric citrate a peculiar behavior of the plants was noticed. The young buds did not separate from the mother frond for several days and grew out at right angles to the surface of the water, protruding into the air. They were almost devoid of color at first but gradually became green, first along the veining and later throughout the entire frond. At the same time the plant resumed a normal position and finally separated from the mother frond by an unusually long strand. Later experiments showed this peculiar growth to occur only in concentration of liver extract above 75 p.p.m. The higher concentrations of iron (32 p.p.m.) did not show the toxic effect of liver extract; the plants were of a medium size and dark green.

In a non-sterile soil suspension to which 300 p.p.m. of liver extract was added (640) the plants became much larger than any yet observed and were a deep green with long heavy roots. The growth constant K of the plants was 16.0 or the generation time was 1.9 days, as compared to a generation time of 5.0 days for the sterilized culture (642). This flask, however, was later found to be contaminated. The plants in cultures in which iron was omitted (646) grew fairly well for a few days, but more slowly near the end of the experiment. The plants were medium size and green showing that liver extract does furnish some iron available for the growth of the plants. Adding 150 p.p.m. of glucose to the above culture produced very small plants with brownish centers, showing a decided toxic effect. A solution containing 300 p.p.m. of liver extract and 32 p.p.m. of iron added as iron citrate was sterilized by means of a Berkfeld filter (656). The plants turned yellow-green and lost roots. Seemingly sterilization by this method removed most of the iron even when added as iron citrate.

Since it was desired to know the effect of liver extract on non-sterile cultures a series of sterile and non-sterile cultures (777-784) were changed every two days. In the non-sterile cultures containing 0.5 p.p.m. of iron (added as the chloride or citrate)(777, 779) and in one containing 5.0 p.p.m. iron (as $FeCl_3$), the plants grew almost

as large as those grown in the soil (801) suspension to which liver extract had been added. They were in all cases dark green and possessed long heavy roots. When 32 p.p.m. of iron (ferric citrate) was added (793) the plants showed no stimulation over the sterile culture (784) nor was the appearance or growth rate in either of these better than plants grown in similar cultures without liver extract. Seemingly liver extract could not stimulate the growth of plants which had a highly available source of iron. Plants in sterile cultures (778, 780, 782) with low iron content were decidedly inferior to the non-sterile. They were medium size, pale color and reproduced at about the same rate as similar cultures without liver extract. A non-sterile culture containing only liver extract as the source of iron (802) reproduced less rapidly than any containing an added iron source; the plants were pale green and showed brown spotting. Glucose added to this solution (803) seemed toxic. Sterilization, by means of a Berkfeld filter (845) of a solution in which liver extract was the iron source produced very poor growth; the plants became small, yellow and lost roots.

Solutions (804 and 808) containing 32 p.p.m. of iron (added as ferric citrate), 300 p.p.m. of liver extract, 50 p.p.m. of glucose and 50 p.p.m. of levulose produced very rapid growth and large green plants. The growth rate was

slightly higher in the sterile (808) culture but the plants were not as healthy in appearance.

From the above experiments it can be seen that liver extract will stimulate markedly growth and reproduction in Lemna under non-sterile conditions but will not do so under sterile conditions. This checks the work of Clark and Roller (1931) on other organic extracts. This stimulative effect was noted only in solutions containing a low concentration of iron and was greatest in a solution containing 0.5 p.p.m. iron as $FeCl_3$. No stimulation could be found in the solutions containing 32 p.p.m. of iron as ferric citrate. It appears, therefore, that the effect of liver extract is upon the availability of iron in these solutions and that where a highly available source of iron is present the stimulation of growth by liver extract does not appear. The effect of sterilization is either to reduce the ability of the liver extract to make iron more available or to produce toxic substances by decomposition of the organic matter. Liver extract alone is a poor source of iron for the growth of Lemna. No consistent effect of manganese could be found in these cultures.

An interesting effect was noted when equivalent portions of glucose and levulose were added to solutions containing a high amount of ferric citrate in addition to the

liver extract. Although no stimulative effect of liver extract had been noticed before in this solution, the presence of the sugars produced much better growth in both sterile and non-sterile cultures than where liver extract alone was added.

Liver extract does not affect the loss of chlorophyll by Lemna when these plants are placed in the dark. The rate of loss of color depends more upon the concentration and availability of the iron which is added to the culture. Thus the rate of loss is greatest in the solution containing the lower concentration of ferric chloride and least in the solution containing the higher amount of ferric citrate. This is just opposite to the effect of non-sterile cultures grown in the light; there, liver extract produced the greatest growth in solutions containing the lower concentrations of $FeCl_3$.

A striking effect is noted with the bacto-peptone agar cultures. These contained 32 p.p.m. of iron (as ferric citrate) and 1 per cent bacto-peptone agar. The plants after 21 days in the dark were still a deep green although no reproduction had taken place. Plants in similar cultures with or without liver extract, which were dark green at the beginning of the experiment, were of a pale green color. That this substance should prevent loss of chlorophyll in the dark, which liver extract appears unable to do, is decidedly interesting.

GLUCOSE

It was noted in the preceding experiment that liver extract appeared to affect the availability of iron. Much better growth was produced by adding liver extract to non-sterile solutions containing low amounts of ferric citrate or ferric chloride, but no stimulation of growth occurred when liver extract was added to a solution containing a high concentration of ferric citrate, i.e., a solution in which the availability of iron is not a limiting factor. That liver extract added to sterile solutions produced slightly less growth than similar solutions without liver extract, suggested either a loss of power to render iron available or production of toxic substances by autoclaving. It was thought that a pure carbohydrate, glucose, should react qualitatively like liver extract if the effect of organic matter is solely upon the availability of iron.

Riffard (1874) developed a method of analyses of sugars which depended upon the prevention of the precipitation of iron by sugar when $FeCl_3$ is added to an ammoniacal solution of the sugar. That plants may excrete reducing sugars from their roots was shown by Knudson (1920). Knudson (1919) has shown also that plants may absorb sugars and show some increase in weight over those in solutions not containing sugar. Albino corn plants lived longer on sugar solutions. Plants

placed in the dark lived longer in Pfeffer's solution to which sugar had been added; they maintained weight, while those in Pfeffer's solution lost 50 per cent of the original dry weight. Continued growth in the dark could not, however, be maintained on the sugar solutions. Molliard (1910) found that radishes would grow in a sucrose or glucose solution. Watercress would grow alone in glucose but would not grow in sucrose unless associated with the radish. Roach (1928) showed that soil algae which would grow independently of organic matter in the light grew on glucose in the dark and was still green after 7 weeks.

EXPERIMENTAL

Merck's C. P. glucose was used in the following experiments in concentrations of flucose from 5-200 p.p.m. The cultures are described in Tables IX and X. For each culture placed in the light a corresponding one was placed in the dark. The experiments were run for the same length of time as those described for liver extract. Later, a series of cultures in the light were changed every two days in order to study the effects of glucose under non-sterile conditions. These are described (785-800) at the end of Tables IX and X, and the growth curves are given in Figure XIII.

TABLE NO. IX

GROWTH OF LEMNA IN CULTURE SOLUTIONS TO WHICH GLUCOSE WAS ADDED

Number of Culture	Source of Iron	Conc. of Iron Mgms./L.	pH	Conc. of Mn Mgms./L.	Treatment of Cultures. (n-s indicates non-sterile) (CLTA indicates grown in constant light and temperature apparatus)	Growth K x 100
681	$FeCl_3$	5.0	4.5	1.0	plus 200 ppm. glucose; sterile; CLTA	10.5
682	"	"	"	"	" " " " n-s "	8.0
683	"	"	"	"	" 100 " " sterile "	9.3
684	"	"	"	"	" " " " n-s "	7.2
685	"	"	"	"	" 50 " " sterile "	12.4
686	"	"	"	"	" " " " n-s "	3.7
687	"	"	"	"	" 5.0 " " sterile "	9.3
688	"	"	"	"	" " " " n-s "	4.2
729	Ferric Citrate	32.0	7.8	"	" 200 " " sterile "	10.8
730	"	"	"	"	" " " " n-s "	9.7
731	"	"	"	"	" 100 " " sterile "	8.1
732	"	"	"	"	" " " " n-s "	9.0
733	"	"	"	"	" 50 " " sterile "	8.4
734	"	"	"	"	" " " " n-s "	8.6
735	"	"	"	"	" 5.0 " " sterile "	8.0
736	"	"	"	"	" " " " n-s "	9.2
740	$FeCl_3$	5.0	4.5	"	" 100 " " sterilized by use of a Berkfeld filter; grown in CLTA	7.8

(Note: The above solutions were not changed. The solutions listed below were changed every two days.

TABLE NO. IX (continued)

GROWTH OF LEMNA IN CULTURE SOLUTIONS TO WHICH GLUCOSE WAS ADDED

Number of Culture	Source of Iron	Conc. of Iron Mgms./L.	pH	Conc. of Mn Mgms./L.	Treatment of Cultures. (n-s indicates non-sterile) (CLTA indicates grown in constant light and temperature apperatus)	Growth K x 100
785	$FeCl_3$	0.5	4.8	0.2	plus 200 ppm. glucose, n-s	CLTA: 5.0
786	"	"	"	"	" " " " sterile	" : 7.6
787	"	"	"	"	" 50 " " n-s	" : 5.5
788	"	"	"	"	" " " " sterile	" : 5.2
789	"	5.0	"	"	" 200 " " n-s	" : 5.2
790	"	"	"	"	" " " " sterile	" : 8.5
791	"	"	"	"	" 50 " " n-s	" : 6.0
792	"	"	"	"	" " " " sterile	" : 9.2
793	Ferric Citrate	0.5	"	"	" 200 " " n-s	" : 5.4
794	"	"	"	"	" " " " sterile	" : 7.5
795	"	"	"	"	" 50 " " n-s	" : 6.1
796	"	"	"	"	" " " " sterile	" : 7.8
797	"	32.0	7.8	"	" 200 " " n-s	" :10.7
798	"	"	" *	"	" " " " sterile	" : 9.0
799	"	"	"	"	" 50 " " n-s	" :11.7
800	"	"	"	"	" " " " sterile	" : 8.8

TABLE NO. X

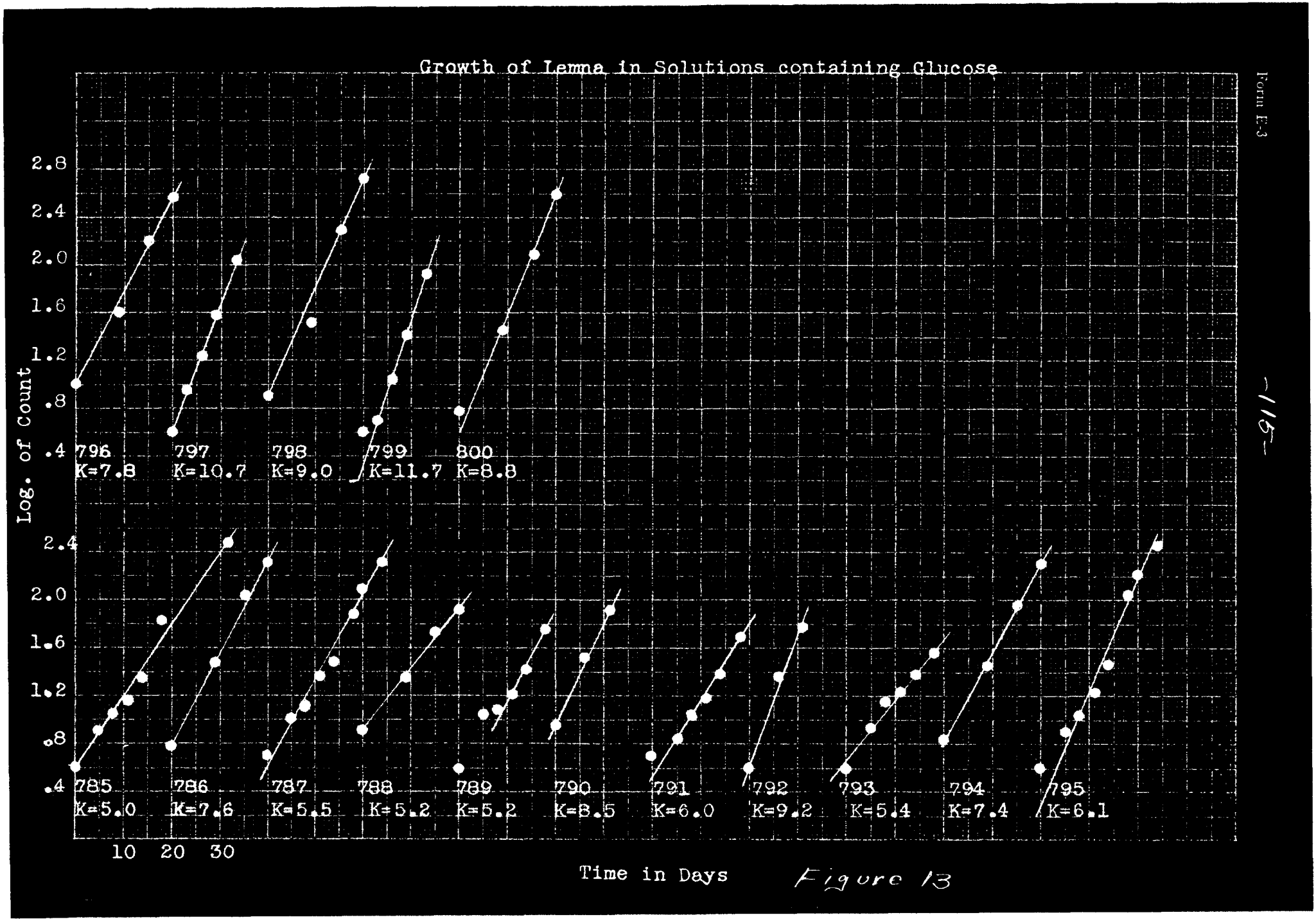
DESCRIPTION OF APPEARANCE AND BEHAVIOR OF LEMNA IN CULTURE SOLUTIONS TO WHICH GLUCOSE WAS ADDED

Number of Culture	Source of Light	Description
681	CLTA	medium size; green; lost roots from overcrowding.
682	"	" " " " algae present.
683	"	large; green.
684	"	medium size; yellow-green; algae abundant.
685	"	" " green.
686	"	large; medium green; algae present.
687	"	medium size; green.
688	"	medium size; pale green; algae present.
729	"	large; green.
730	"	" "
731	"	" "
732	"	" "
733	"	medium; green.
734	"	large; green.
735	"	medium; pale green; lost roots; brown centers to fronds.
740	"	small; yellow; dying.
785	"	medium size; yellow-green; lost roots.
786	"	" " " " " " reddish tinge.
787	"	small " yellow; lost roots.
788	"	medium size; yellow-green; lost roots; reddish tinge.
789	"	" " " " " " " "
790	"	" " " " " " " "
791	"	" " " "
792	"	large; pale green; white tips on fronds.

TABLE NO. X (continued)

DESCRIPTION OF APPEARANCE AND BEHAVIOR OF LEMNA IN CULTURE SOLUTIONS TO WHICH GLUCOSE WAS ADDED

Number of Culture	Source of Light	Description
793	CLTA	medium size; pale green; lost roots; white tips.
794	"	large; medium green; lost roots; reddish tinge.
795	"	large; " " few brown spots.
796	"	medium; green; lost roots.
797	"	" "
798	"	" "
799	"	" "
800	"	" "



Form E-3

-116-

Figure 13

DISCUSSION OF EXPERIMENTAL RESULTS

The cultures which were placed in the dark showed no reproduction at the end of 9, 11 and 21 days for different experiments. The color of the plants in solutions containing glucose was no different from those of the control or from those growing in solutions containing liver extract. Here, as noted with liver extract, the rate of loss of color was greatest in solutions containing 5.0 p.p.m. $FeCl_3$ and lowest in the solutions containing 32 p.p.m. of iron as ferric citrate. Apparently Lemna major is not able to assimilate glucose in the dark, nor does glucose prevent loss of chlorophyll from these plants.

Of the cultures (681-688, 729-736, 740) grown in light for 9 days without change, only the sterile ones are significant. Algae were very abundant in the non-sterile cultures and in some of the flasks the plants were dead. In all cases the sterile cultures showed the higher growth rate. No relation of the concentration of glucose to rate of reproduction could be found in either the sterile or non-sterile cultures, although in the sterile cultures a concentration of 50 p.p.m. gave larger and greener plants in the solutions containing 5 p.p.m. of iron as $FeCl_3$. The growth rate of the sterile cultures to which glucose was added was approximately that of similar solutions containing no glucose.

Very little information could be drawn from the above experiment; a new set, therefore, of sterile and non-sterile cultures were changed to fresh solutions every two or three days.

The description of these cultures (785-800) is given in the latter part of Tables IX and X. Growth curves are given in Figure XIII.

In this group the sterile cultures reproduced faster than the non-sterile in all cases except where 32 p.p.m. of iron as ferric citrate was present in the solution. In this solution the non-sterile plants reproduced faster but were no better in appearance. Also, the non-sterile cultures showed slower reproduction and were poor in appearance when compared with the controls receiving no glucose. In the non-sterile cultures 50 p.p.m. of glucose gave faster reproduction than 100 p.p.m. In the sterile cultures no correlation of concentration and growth could be made. The growth rate of the sterile cultures were slightly less than the controls in all cases.

It can be seen from the results of these experiments that the effect of glucose is entirely different from that of liver extract. Liver extract gave the highest rate of reproduction in non-sterile cultures in all cases and produced large green plants in solutions containing 0.5 p.p.m. $FeCl_3$

and ferric citrate. In all solutions the plants possessed a green color. Glucose, on the other hand, produced growth in sterile cultures almost identical with the controls, was toxic in non-sterile cultures and did not affect appreciably the size and greenness of the plants.

It appears, therefore, that glucose does not make iron more available to Lemna, is toxic in non-sterile cultures and of little effect in sterile solutions. The work of other investigators who claim sugars may be assimilated by green plants is in sharp contrast with the results obtained here with Lemna major.

CONCLUSIONS

It is recognized that the role of iron in the manufacture of chlorphyll, and in the composition of the oxidizing enzyme catalase, makes the problem of its availability to plants of primary importance. The work reported here offers further evidence of the effect of organic matter upon the availability of iron to plants grown in nutrient solutions.

Evidence from several sources shows that inorganic iron becomes quickly unavailable to plants when the hydrogen ion concentration of the medium is brought to the neutral or alkaline point, but that organic iron furnishes the plants with iron when the pH is increased. Emerson (1929) and Hopkins (1930) show that increasing the iron concentration of the medium increases the growth of plants. That there is an optimum pH for growth for each concentration of iron added, and that this optimum pH grows larger with increasing amounts of iron, has not been shown previously. For Lemna the optimum pH does not rise above 7.8 however much iron is added. This, however, is the maximum reproduction rate obtained, and it may therefore be assumed that when the availability of iron is not a limiting factor plant growth is better in a neutral or alkaline medium.

Previous investigators, who have reported on the properties of ferric citrate and other organic iron salts,

vary considerably in their explanation of the mechanism by which iron is furnished to the plant. The reduction of iron salts by light and the consequent slow reoxidation and precipitation of the hydroxide favors the theories of Thomas (1929) and Hopkins (1930). The slowness with which final equilibrium is reached in the various phases (solution, sol, and precipitate), the reactions and changes in composition which take place during the precipitation of the iron, and the influence of pH and organic substances upon these phenomena make the problem of iron availability difficult to investigate.

Manganese is found in these experiments to be non-essential for the growth of Lemna; this contrasts with the reports of many investigators on the essential nature of manganese for a number of plants. Manganese does not increase the rate of reproduction of Lemna in inorganic solutions but, when present in optimum concentrations, tends to increase the size and greenness of the plant. Its action is thus quite different from that of iron; the correlation between iron concentration and rate of reproduction of Lemna has been well established. If the action of manganese is to promote the oxidation of ferrous to ferric iron, as suggested by Hopkins (1930), the toxic effect of manganese in neutral and basic inorganic solutions may be explained. It is shown by Fischer and others (1931) that the iron porphyrin complexes, which

are the basis for the formation of hemin and catalase, cannot form unless the iron is in a ferrous condition. A high concentration of manganese, an alkaline reaction and strong light would tend to keep the iron in an oxidized state, thus decreasing the ability of the plant to synthesize chlorophyll and catalase. The reducing action of organic matter on iron must offset this effect or chlorosis will occur. It is exactly under these conditions: low organic matter, neutral or alkaline soil and strong sunlight that manganese-induced chlorosis does appear. The fact that manganese was less toxic in the solutions containing organic matter is also explained by this theory.

Liver extract and glucose give conflicting results if the sole effect of organic matter in nutrient solutions is considered to be upon the availability of iron. Neither of these substances could produce growth of Lemna in the dark or reduce the rate at which chlorophyll was lost. In the light, liver extract proved to be a very powerful stimulant for both size and rate of reproduction in the non-sterile cultures; on the other hand glucose reduced growth in the non-sterile cultures even in low concentrations. In the sterile cultures, liver extract reduced the rate of reproduction while glucose appeared ineffective. Bacto-peptone agar prevented the loss of chlorophyll by sterile plants

placed in the dark. Such a wide qualitative variation suggests other functions of organic matter than that of effecting the availability of iron.

SUMMARY

1. Technique and apparatus are described for the study of the growth of plants in nutrient solutions. Sterile and non-sterile Lemna major (Spirodela polyrhiza) were used in these investigations.

2. When inorganic iron was used as the source of that element in Clark's nutrient solution, the optimum pH range was 4.5-4.8.

3. Toxicity of manganese was noticed in concentrations of 1 p.p.m. and above; this toxic effect was increased in the more alkaline solutions in strong light.

4. Sterile inorganic cultures reproduced faster and were of better appearance than non-sterile ones.

5. The properties of ferric citrate in water and culture solutions were studied. The compound was found to decompose in nutrient solutions in the light with almost complete precipitation of the iron.

6. Data was obtained upon the buffering power of ferric citrate in Clark's nutrient solution.

7. The results of attempts to prepare a ferric citrate of definite concentration cast serious doubt upon the reliability of the method suggested by Belloni for this purpose.

8. With organic iron asthe source of that element

the growth rate of Lemna for any definite pH, up to a certain limit, was increased by added amounts of iron. For each concentration of iron an optimum pH was observed; the optimum pH increased with increasing amounts of iron up to a value of 7.8, which seemed maximum for the best growth of Lemna. (Stunted plants were obtained in concentrations of iron above 64 mgms./liter.)

9. Manganese lowered the rate of reproduction in the non-sterile cultures; it slightly improved the rate of growth in the sterile cultures.

10. Sterilization of solutions containing organic matter lowered the rate of reproduction of Lemna in all cases.

11. Liver extract does not prevent the loss of chlorophyll by Lemna when that plant is placed in the dark. It does, however, stimulate greatly the growth rate and the size of non-sterile (but not of sterile) plants grown in the light.

12. Bacto-peptone agar was observed to prevent loss of chlorophyll by Lemna; the plants were dark green after 21 days in the dark.

13. Glucose did not stimulate the growth of Lemna major under any conditions; it was ineffective in the sterile, and toxic in the non-sterile cultures.

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