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Effect of plant growth-promoting substances on vitamin content and reproduction of Lemna

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NOTE TO USERS

EFFECT OF PLANT GROWTH-PROMOTING SUBSTANCES ON VITAMIN CONTENT AND REPRODUCTION OF LEMNA.

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INTRODUCTION

The effect of minute traces of organic compounds on plant growth has been a subject for investigation for a number of years. Organic matter seemed to be a necessary addition to inorganic media in order to secure normal plants. Healthy and normal growth in inorganic media was reported as impossible and, therefore, organic matter was supposed to contain substances essential for the growth of plants. Later, the essential character of these substances was questioned, and organic matter found to be more of a stimulant than an essential. Healthy green plants were grown and shown to reproduce in inorganic media, but the question of how the organic matter stimulated the plants remained unsolved.

Recently, green plants were found to contain traces of certain organic acids termed " auxins " ; these are produced in the plants themselves and play an important part in growth. The growth substances were first thought to be few in number and specific in their action, but a large number of synthetic organic acids, their esters and salts, and even some organic gases, were discovered, which produced effects in plants similar to the original auxins. Finally, other naturally occurring organic compounds, including some of the vitamins (essential constituents of the animal diet) were added to the

ever increasing number of plant growth-promoters.

The number and variety of effects produced in plants by growth substances have multiplied. Early investigations were concerned with the production of curvature and elongation of the coleoptile and roots of grasses, especially Avena; later experiments were related to the production of abnormal conditions, for example, the production of adventitious roots on the upper stems and branches of complete plants. More recent investigators have questioned whether substances which produced such effects could be classed as growth substances; they seemed, under these conditions, to be detrimental to the plants. Investigations in which the normal healthy condition of the plants is maintained, show that some of the synthetic growth promoters not only fail to stimulate plants, but also that they are toxic, outside a narrow range.

This thesis is a report on the effect of various organic substances on the growth, reproduction and composition of the plant Lemna major (Spirodella polyrhiza).

HISTORY.

Of the early work on the stimulation of plant growth by organic matter, the investigations at the Bureau of Soils at Washington, D. C., are outstanding. Schreiner and Lathrop(61) and Schreiner and Skinner (62) extracted and isolated from soils a number of organic compounds, some beneficial, others

extremely toxic to wheat seedlings when added to nutrient solutions. Bottomley (5) of the University of London, was unable to secure normal growth of Lemna (duckweed) in a pure inorganic medium; he found organic matter, especially in the form of well-rotted peat or peat extracts, was a necessary constituent of a nutrient solution promoting healthy growth of Lemna. Bottomley concluded that organic matter contained substances, which he called "auximones", essential for the growth of plants. Mockeridge (53), a co-worker of Bottomley, suggested that auximones might consist of nucleic acid and its derivatives and these were shown to be constituents of the organic materials investigated; Mockeridge (54) from results of later experiments, further suggested that soil micro-organisms were instrumental in the formation of plant growth-promoting substances from organic residues in the soil. The essential character, however, of the auximones of Bottomley and Mockeridge was disputed by other investigators.

Clark (11) and Clark and Roller (16,17) successfully grew Lemna major in a completely inorganic medium consisting of recrystallized inorganic salts and water. The plants were healthy and reproduced normally; but these investigators found that the addition to their medium of organic matter in the form of extracts of manure and other organic materials, did increase the rate of reproduction of non-sterile plants grown under non-sterile conditions. However, the same organic matter

did not benefit sterile Lemna under sterile growth conditions. Saeger (59) confirmed the results of Clark and Roller by growing Lemna in a diluted Knop's solution, finding no decrease in the rate of growth of the plants in the inorganic solution, but when either autolyzed yeast or alkaline peat extracts were included in the solution, a considerably increased growth rate resulted. Later, Saeger (60) reported normal reproduction of Lemna in the same solution in the absence of microorganisms; the addition of an extract of autolyzed yeast under these conditions also increased the rate of growth of the plants. Wolfe (80) although growing Lemna minor successfully in Shive's inorganic medium, failed to get any increase in reproduction when he added a number of pure organic compounds to the nutrient solution. Ashby (1), using the inorganic medium of Clark (13), secured normal healthy growth of Lemna minor and also reported a marked stimulation in the growth rate upon the addition of organic matter to the non-sterile solution.

These investigations showed that certain types of organic matter, including Bottomley and Mockeridge's auximones, were not necessary for normal growth of plants but could be characterized as accessory growth factors if added to non-sterile nutrient solutions. Saeger (60) however, also secured stimulation under sterile growth conditions. Meanwhile, the problem had been attacked from another point of

view and experiments with plant growth-promoting substances, produced and functioning in plants themselves, were in progress.

The early work on growth-promoting substances produced in plants was done on the primary leaf sheath or coleoptile of grasses, especially Avena. Boysen-Jensen (6) found that the removal of the coleoptile tip destroyed the power of the coleoptile to respond to light; restoration of the tip to the decapitated coleoptile restored this power. Paál (56), secured indications that the tip of the coleoptile normally produces a growth accelerating substance. Paál cut off the tips of coleoptile of Coix Lacrima and replaced them eccentrically upon the coleoptile stumps; the coleoptile curved away from the side covered by the tip and this he supposed was caused by a growth-accelerating substance which passed from the tip to that side of the coleoptile. Söding (65), from similar experiments with oat coleoptiles, also concluded that the coleoptile tips formed such an accelerating substance. Definite evidence of this substance was secured by Went (75), who extracted it by placing the severed coleoptile tips on agar blocks. The agar blocks were then placed on freshly decapitated coleoptiles; curvature away from the block was secured, similar to that recorded by Paál and Söding for the eccentrically replaced tips. Kögl, Erxleben and Haagen-Smit (38), named this stimulating sub-

stance "auxin" and later, isolated two auxins from plant materials; they found corn oil contained a comparatively large amount of the two substances and designated them auxin a and auxin b. Both of these were found to cause curvature of decapitated oat coleoptiles. Kögl and Erxleben (37), determined the constitution of these two auxins.

Using human urine as a source of the auxins, Kögl, Haagen-Smit and Erxleben (40), discovered still another auxin existing together with auxin a and auxin b. This was named heteroauxin and identified as beta-indolyl acetic acid. Kögl, et al (42) isolated this auxin from yeast and from cultures of the molds Rhizopus nigricans and Aspergillus niger. Along similar lines, Nielsen and Hartelius (55), found Rhizopus suinus produced two growth-promoting substances; one of these substances accelerated the growth of another mold, Aspergillus niger, while the other did not, but did cause bending of oat coleoptiles. Thimann (68) isolated the growth-promoting substance produced by Rhizopus suinus which caused the bending of the oat coleoptiles; it also proved to be heteroauxin or beta-indolyl acetic acid.

From 1930 investigations on the identity and physiological action of plant growth-promoting substances found in plants rapidly increased and the substances were shown to have an active part in the life processes of plants. At

first they were thought to be few in number, but this assumption was soon disproved.

A series of investigations was carried out at the Boyce Thompson Institute with a wide range of organic compounds. Zimmerman and Wilcoxon (83) performed experiments on African marigold, tomato, buckwheat and other plants; water solutions of the compounds were injected into the plants or applied externally to the stems or leaves in the form of a paste in lanolin. Compounds used were mainly indole derivatives, including beta-indolyl acetic acid and a number of substituted fatty acids. Results recorded were initiation of adventitious roots, bending of plant stems and leaf petioles, intumescence and epinasty and hyponasty of leaves. Following this, Hitchcock and Zimmerman (34) poured water solutions of indole derivatives and naphthalene and phenyl substituted fatty acids directly on the soil in which tomato and tobacco plants were grown; qualitative results obtained were similar to the previous experiments. Zimmerman and his co-workers, (81,82) using potted plants, found the salts of the organic acids to be generally more effective than the free acids; in some cases esters were found to be more effective than the free acid, for example, methyl-beta-indolyl acetate gave the same results in lower concentration as the synthetic heteroauxin, beta-indolyl acetic acid.

Avery, Burkholder and Creighton (2) used the Avena

coleoptile as a test object and reported results of experiments with a number of synthetic compounds found by the investigations at the Boyce Thompson Institute to be highly effective growth substances. On the basis of minimum concentrations which caused curvature of the coleoptile of about ten degrees, the salts and esters were found to be of equal or greater effectiveness as growth substances when compared to the free acids. Two acids, naphthalene acetic acid and indolyl butyric acid, both powerful root-forming substances, appeared to be only slightly effective on Avena. This report showed that acids which had been found effective as growth substances can have the hydrogen of the carboxyl group replaced by an organic or inorganic radical or a metal without destroying the activity.

A completely different type of growth stimulating substance was found in gases; Crocker, Hitchcock and Zimmerman (19), using tomato plants and sweet-pea seedlings, reported plant growth-promoting powers for the gases carbon monoxide, ethylene, acetylene, propylene and butylene, comparable to those of the acids, esters and salts studied. Denny (21) discovered that tomato stems produce an emanation which induced epinasty of potato leaves in a manner similar to low concentrations of ethylene. Michener (52), reached the conclusion that the effects of ethylene on growth were not entirely direct, but that the ethylene also influenced

the growth hormones.

The number of growth substances, increased by the synthetic compounds discovered by experiments at the Boyce Thompson Institute, was further augmented by the addition of fresh naturally occurring substances active as growth-promoters.

Williams and others (77), discovered a growth-promoting substance which they extracted from beef liver, rice bran, sea urchin eggs, molds and bacterial growth; this appeared to be a constituent of every organic tissue examined, whether of animal, plant or bacterial origin. The substance was isolated in a concentrate pure enough to be classed as an organic acid and was named pantothenic acid; although a large part of the investigations of pantothenic acid as a growth substance, by Williams and his co-workers, was on yeast, McBurney, Bollen and Williams (48) reported the acceleration of the growth of alfalfa seedlings by the acid.

Vitamins, essential constituents of animal diet, have also been found to be plant growth-promoting substances. van Hausen (32) reported that the addition of pure Vitamin C to sterile pea cultures caused an increase of 35 to 70 per-cent in dry weight of the plants over controls. Vitamin C, contained in cotyledons of the pea seedlings, was found to exert a decisive influence on the development of young plants; while removal of the cotyledons almost completely checked the growth of the seedlings, the addition of

vitamin C to the nutrient solution resulted in nearly perfect development of the plants and these later contained normal vitamin C content. Virtanen (71), from similar experiments concluded that vitamin C was a phytohormone which was indispensable to plants. In the same way, vitamin B_1 is indicated as an essential in plant growth; Bonner (3) was unable to grow amputated pea roots in solutions containing essential mineral salts and sucrose; new growth was obtained when an extract of yeast was added to the nutrient solution and still better growth when crystalline vitamin B_1 was included with the basal medium. Bonner concluded that vitamin B_1 was as necessary for plant growth as it is for the normal growth and health of animals.

Thus the number of known compounds which promote the growth of plants was increased. To the auximones of Bottomley and Mockeridge and the auxins of Went, Kögl and Thimann, were added many organic acids, their esters and salts, vitamins and even gases. Following the discovery at the Boyce Thompson Institute of an increasing number of synthetic organic compounds (a total of 53 were reported by Zimmerman and Hitchcock (81)), which were efficient plant growth-promoting substances, Zimmerman and Wilcoxon (83) made the following statement regarding the plant growth substances:

"Of the many thousands of known chemical compounds there are probably many others which would be equally effective with those known to date".

The data of Zimmerman and Hitchcock (81) argue against the idea of the specificity of a particular hormone or growth substance for any one plant, and Söding (66) also has reported experiments from which he concludes that growth substances are non-specific.

In the search for plant growth-promoting substances the Avena coleoptile has been used widely as a test object. To obtain reliable results with the method developed, it is necessary to use a standarized strain of oats and to maintain a constant temperature in a dark room with high humidity. Only one of several growth responses induced by growth substances is measured, namely, the elongation of cells. The Avena method does not measure cell enlargement, cell division, penetration of the substance or induction of new organs.

A technique developed at the Boyce Thompson Institute by Zimmerman and Wilcoxon (83), makes use of the tomato plant, although many other species were also used. The growth substances are mixed with lanolin or olive oil and applied to stem, leaves, etc., of the plants. Water solutions of the substances can be injected into the plant, added to the soil in which the plants are grown, or used as a nutrient solution,

and the responses of plants to the growth substances can be investigated and measured. Although these methods show many of the effects on plant growth, experiments investigating the effect of growth substances during the entire life cycle of the plant seem to be limited to the growth of peas, carried out in the absence of microorganisms by van Hausen and Virtanen.

In this laboratory, Lemna major had been grown successfully through hundreds of plant generations under artificial light, in an inorganic medium and in the absence of microorganisms. Under these growth conditions Clark and Roller (16,17) found that organic matter from soil did not stimulate the rate of reproduction of the plants. The healthy and normal growth of Lemna under sterile growth conditions and in the absence of organic matter, offered an opportunity to investigate the effect of pure or crude concentrates of growth substances on one plant, in its complete form and under its natural means of acquiring nutrients for growth. The question of the synthesis of vitamin B_1 by the Lemna under these conditions,(Clark 15), was a further problem and remained to be solved.

OUTLINE OF PROBLEM.

1. To determine the effect on the rate of reproduction of Lemna grown in inorganic medium in the absence of micro-organisms and under artificial light of the following:-

   (a) a crude concentrate of growth-promoting material extracted from beef liver (pantothenic acid);

   (b) a crude concentrate of growth-promoting material prepared from the shoots and roots of sprouted corn (auxins);

   (c) pure organic compounds known to be plant growth-promoting substances.

2. To determine if Lemna synthesize vitamin B_1 when grown under the following conditions:-

   (a) in an inorganic medium, in the absence of microorganisms and under artificial light;

   (b) in a soil-water mixture, in the presence of microorganisms and under sunlight.

The first crude concentrates of growth substances extracted from dry beef liver by 80 percent methyl alcohol and from the shoots and roots of sprouted corn by 95 percent ethyl alcohol, were further concentrated by the use of the fractional electrolysis method of Williams and Truesdail (78).

Of the pure compounds used, phenyl acetic acid and phenyl propionic acid were purchased from the Eastman Kodak Company; beta-indolyl acetic acid (synthetic heteroauxin) was obtained through the courtesy of Dr. R. H. Manske of the National Research Council, Ottawa, Canada.

In the investigation of the synthesis of vitamin B_1 by the Lemna, the preparation of the rats and rat feeding experiments were carried out with the aid of Dr. B. H. Thomas and his assistants of the Animal Nutrition Laboratory of the Agricultural Experiment Station.

No attempt has been made in the introduction of this thesis, or in the sections to follow, to review completely the literature on plant growth-promoting substances and vitamins; articles directly related by their nature to the problem undertaken have been cited.

Thimann (69) has published a comprehensive review of the literature on growth substances up to 1935 ; Went, F.W. (75) reviews auxins and Went (74) presents a complete review of the work of the laboratory at the University of Utrecht (Kögl, Went, et al) during the last decade. Boysen-Jensen's (8) book on growth hormones in plants has been revised and translated into English; this is a complete study of plant growth substances up to and including part of 1936.

A review of vitamin B_1 is given by Brady (9);Sherman

and Sherman (63) give an extensive review of recent investigations of vitamins up to 1937.

PANTOTHENIC ACID

The growth promoting substance named pantothenic acid was discovered by Williams and his co-workers (77) in 1933. They extracted this substance from rice bran, beef liver, crab eggs, sea urchin eggs, oysters, earthworms, planarian worms, slime mold, bacteria, (B. subtiles), mold (Aspergillus niger), algae, milk and egg white. The extraction procedure was essentially the same for all raw materials.

The dry substance was extracted for approximately one half hour, under reflux, with 80 percent methyl alcohol. Raw materials which contained much fat were first extracted with ether; the residue after extracting with ether was then treated with the 80 percent methyl alcohol and although the ether extract was always tested for growth-promoting material, none was ever found. The methyl alcohol extract was filtered free of insoluble material, the filtrate evaporated to dryness on a water bath, the residue taken up in water, made up to the required volume and the solution purified by electrolysis.

The electrolysis apparatus used by Williams and his co-workers was patterned after an electrodialysis system suggested by Williams and Waterman (79). It consisted of a series of four cells 5 cm. in diameter and 5 cm. high; the cells were connected by syphons, which were filled by suction

with the same solution contained in the cells and emptied at the end of the electrolysis by opening the stopcocks at the top of the syphons. Platinum flag electrodes, placed in the end cells of the series, were connected to a source of direct current. The apparatus used was similar to that of Williams and Truesdail (78), except that the number of cells was increased from four to eight.

During the electrolysis of water solutions in the apparatus described, a pH gradient was established ranging from acid in the cathode cell to alkaline in the anode compartment. By increasing the number of cells from four to eight, Williams and his co-workers (77) secured a more uniform pH gradient. The growth substance contained in the extracts investigated was found to move toward the anode during an electrolysis. By securing a more uniform pH gradient, a concentrate of greater purity was obtained.

The time of electrolysis was usually from 30 to 50 hours for all materials investigated; a potential of 500 volts was used with a current strength varying from 1 to 6 milliamperes, depending upon the nature of the material.

Williams (77), explains the concentration of the growth substance (pantothenic acid) in certain of the cells by the fact that a weak acid in the presence of buffering substances migrates in an electric field to a point where its ionization will be too low to be effective; as it passes

to cells whose contents are quite acidic the ionization of the acid will be reduced to such a low value that its movement to the next cell will be comparatively slow. If electrolysis were always carried out under the same conditions, the acid should accumulate in the same place; but to duplicate the exact conditions is impossible because the presence of foreign substances influences the result. Williams (77), believed the character of the pH gradient to be determined almost wholly by the substances associated with the pantothenic acid because the concentration of the acid itself was negligibly small.

A striking stimulation of yeast growth was secured when pantothenic acid in the form of a crude concentrate obtained from electrolysis experiments was added to a synthetic medium; this was true regardless of the source of the concentrate. Based on the similarity of behavior in fractional electrolysis experiments, diffusion experiments, and different chemical treatment such as oxidation and hydrogenation, Williams (77) concluded that the ability of these extracts to stimulate yeast growth was due to the presence of a single acid substance which appeared to be of universal biological occurrence.

Although the greater number of the investigations of Williams and his co-workers (77) was on the stimulation of yeast growth by the pantothenic acid, McBurney, Bollen and

Williams (48) reported the stimulation of alfalfa seedlings by the extracts.

In the investigations of naturally occurring growth substances for Lemna in this laboratory the electrolysis procedure developed by Williams and his co-workers seemed to be a promising method for the concentration of growth substances which might stimulate the rate of reproduction of Lemna; beef liver was used as a source of the growth substance, since Rohrman, Burget and Williams (58) found that liver was one of the richest sources of the pantothenic acid.

Growth methods for Lemna.

Fresh beef liver was cut into narrow, thin strips and completely dried in an oven at 50° C. The dried liver was ground in a mortar; the resulting powder was placed in the thimble of a soxhlet extractor and extracted with ether for 30 minutes. The residue was freed of ether and then extracted under reflux with 80 percent methyl alcohol for 45 minutes. The solution obtained was filtered and the filtrate evaporated to dryness on a water bath. The dry residue was taken up in water and diluted to the volume desired for further concentration of growth substances by electrolysis.

The apparatus used for the electrolysis consisted of eight 100 cc. pyrex beakers arranged in a row upon a wooden

platform with cleats on the sides to hold the beakers in line, and covered with a wooden strip. Soft rubber sheeting was tacked to the under side of the board, fitting snugly against the top of the beakers when the cover was clamped in place, in order to reduce loss of solution from evaporation to a minimum. The cover also formed a support for inverted glass U-tubes which passed through holes in the board and were used to connect the beakers with liquid junctions of the same solution contained in the beakers. A small glass tube was sealed into the bend of each U-tube and these were closed at the top by means of short pieces of rubber tubing fitted with Hoffman clamps. The rubber tubing served to fill the tubes by suction. At the end of the electrolysis the tubes were emptied by loosening the clamps. Platinum flag electrodes in beakers at opposite ends of the series were supported by the cover. A 2000 volt Westinghouse high voltage rectifier, commonly called a power pack and operating on 110 A.C., was used as the source of current.

In carrying out an electrolysis each cell (beaker) in the series was filled with 100 cc. of the solution to be electrolyzed and the cover clamped in place; the U-tubes were filled by suction, the electrodes placed in the end cells, connected to the power pack and the current turned on. The electrolysis was continued without interruption for as long a period as was desired. At the end of the electrolysis the

liquid junctions were emptied, the cover and electrodes removed, and the pH of the contents of the separate beakers was determined with a glass electrode. Aliquots of the contents of the cells were then removed and added to a basal inorganic solution used as nutrient solution for Lemna.

The basal inorganic media used was identical in all variations of the investigation. This solution was described by Clark (13) and found by him to give optimum reproduction of Lemna major at pH 4.8. The prepared basal solution contained, in millimols per liter, 0.4 of calcium added as mono-calcium phosphate, 8 of potassium as potassium nitrate, 1 of magnesium as magnesium sulfate, 0.00046 of manganese as manganous chloride, and 0.01 of iron as ferric chloride. In addition, each liter of nutrient media contained 2 cc. of a 0.0285 molar ammonium chloride solution.

All the salts used were purified by recrystallization, except ferric chloride; this salt was Kahlbaum's "Zur Analyse mit Garantieschein".

Special distilled water was used to prepare all solutions. This was prepared from regular laboratory distilled water which was redistilled with alkaline permangenate. The product of the second distillation was further distilled in an apparatus constructed entirely of pyrex glass. The special distilled water was stored in a 20 liter pyrex carboy and protected from contamination by dust. Pyrex apparatus

was used throughout and containers with which the culture solution came in contact were also pyrex.

The aliquots of stock solutions of the inorganic salt used in preparation of the basal inorganic medium were added from burettes to a 100 cc. pyrex volumetric flask; the desired aliquot of supplementary material was then transferred to the flask with a graduated pipette. The volume of the mixed solution was made up to approximately 90 cc. with special distilled water and the mixture transferred to a 250 cc. pyrex beaker. The pH of the solution was adjusted to 4.7 to 4.8 by the addition of either a dilute solution of hydrochloric acid or of potassium hydr oxide, depending on the original pH of the solution; measurements of pH were made with a glass electrode. Following adjustment of the reaction of the nutrient solution, it was returned to the volumetric flask and the volume made up to 100 cc. with special distilled water.

The prepared media was transferred to a 250 cc. erlenmeyer flask, stoppered with a cotton plug and sterilized in a steam autoclave at 20 pounds for 15 minutes. Williams and others (77), reported the growth substance to be heat stable in slightly acid solutions, so the treatment given the supplemented media should not have destroyed any of the added substances.

Lemna plants, which had been grown under sterile con-

ditions on inorganic medium for a number of years in this laboratory, were used to inoculate the sterile cultures. The apparatus used and the technique followed in inoculating and transferring cultures was essentially that described by Clark (14). Sterility of the cultures was checked periodically by inoculation of bacto-nutrient agar slants with one of the plants, as outlined by Clark and Roller (17).

The inoculated nutrient solutions were placed in a constant light and temperature apparatus, described by Clark (14). This apparatus contained a water bath maintained at 25° C ± 0.5°, in which the culture flasks were placed. The light was furnished by four 300 watt bulbs for 14½ hours daily; the intensity of the light was measured by a Weston Illumination meter and found to be approximately 150 foot candles at the surface of the bath.

The rate of reproduction and general appearance of Lemna grown in an inorganic medium, under the conditions described, was taken as a standard for comparison in all investigations recorded in this thesis. The inorganic medium used was the basal inorganic solution, adjusted to a pH of 4.7 to 4.8 with dilute potassium hydroxide and made up to 100 cc. with special distilled water. The procedure in sterilization and the growth conditions were identical with those used for the supplemented basal medium. Hereafter, the appearance and rate of reproduction of Lemna in the

inorganic solution will be referred to as the "standard".

Experimental

Stimulation of Lemna: preliminary tests.

Series I: Test of different concentrations of growth-promoting material.

Preparation of media.

A preliminary investigation was made to test for the presence of substances in dry beef liver which would stimulate the rate of reproduction of Lemna, and also to examine the feasibility of the fractional electrolysis of Williams and others (77), as a method of separating such growth-promoters. The data presented represent only a small part of the results obtained but the general trend of the selected material indicates clearly the answer sought by these first experiments.

The preparation of the supplement for the basal medium was duplicated as nearly as possible for each part of the series. In every instance a five gram lot of dry beef liver was prepared and extracted by the procedure described in the section on "Methods for Lemna". The water solution secured was made up to a volume of 833 cc. ; the excess over the 800 cc. needed to fill the cells of the electrolysis apparatus was used to prepare nutrient solution supplemented with "unelectrolyzed"solution.

The electrolysis of the water solution was continued for approximately 20 hours for each five gram lot of dry liver. The current strength varied at the start of an electrolysis from 5.0 to 8.0 milliamperes; this dropped rapidly after two to four hours from 3.0 to 4.0 milliamperes and then decreased gradually to 1.5 and 2.0 milliamperes at the end of the run. The voltage dropped from approximately 1900 to 1750 volts during the first few hours and then gradually increased from 1900 to 1975 volts at the conclusion of the operation.

At the end of an electrolysis the pH of the contents of the cells was determined with a glass electrode. Aliquots were then removed from the cells by means of a graduated pipette, the measured portion added to the inorganic media and the pH adjusted to 4.7 to 4.8, as previously described. After the reaction was adjusted, the prepared nutrient solution was made up to a volume of 100 cc. and transferred to a 250 cc. erlenmeyer flask, which was immediately stoppered with a cotton plug and sterilized in an autoclave. Results of preliminary investigations showed that the precipitate which accumulated in some of the electrolytic cells at the end of an electrolysis was neither toxic nor stimulative to Lemna ; accordingly, the sediment in the cells was well mixed with the liquid and the aliquots immediately removed from the resulting suspension.

The volume of the aliquot taken from the cells and added to the basal inorganic medium varied from 9 cc to as small a portion as 3/16 cc. Three, and in some instances four and five duplicate cultures were prepared from the material extracted from one lot of liver. This procedure was necessary because the preparation of the material and the nutrient solutions (extractions of liver, electrolysis of extracts and adjustment of the pH of cultures) involved a time-consuming amount of routine work. Since the pH adjustment of supplemented solutions was different from the material prepared from each lot of liver extract, repetition of that part of the preparation was avoided by preparing the cultures in the number of duplicates mentioned. Unless otherwise indicated, the cultures were transferred to fresh solutions every five days; this method necessitated using supplemented media which had been reserved in the laboratory several weeks before inoculation with Lemna. There was no evidence of loss of growth-promoting power or of the development of substances toxic to Lemna in the solutions during the interval before use. In order to continue an experiment for four to five weeks, material was required from the electrolysis of extracts of at least two separate lots of dry liver. The cultures used in the two parts of the experiment were prepared from similar material. For example, if the aliquots added during the first part of the experiment were taken from

a cell in the electrolysis apparatus whose contents had a pH of 3.4, then the inorganic solution used to continue the series was supplemented with a similar aliquot from the cell in the second electrolysis with a pH nearest 3.4. The variation in the composition of the cultures caused by this procedure is indicated in the tables and graphs.

The results of the electrolysis of nine separate lots of liver are summarized in Table I. Selected data from preliminary experiments on the growth of Lemna in inorganic media containing an extract of beef liver, are recorded in Table II; the curves in Figure I, show the rate of reproduction of the cultures described in Table II.

In these investigations, the terms "growth" and "rate of reproduction" are used interchangeably. Lemna major reproduces by budding. When conditions suitable for growth are kept constant, the rate of reproduction, K, may be calculated by using the equation derived by Clark (12), $\log_{10}N - \log_{10}N_o = K\,(t-t_o)$, in which N is the number of fronds at any time, t. The rate of reproduction is determined graphically by plotting $\log_{10}N$ against the time in days, t ; the slope of the resulting curve represents the rate of reproduction, K. The curves, for the cultures described in Table II, are plotted in Figures 1 and 2.

Table I.- Fractionation by electrolysis of water solution of extract of beef liver.

Cell Number	pH of cells (glass electrode) at conclusion of electrolysis. Range of pH nine runs	Average pH nine runs	Sample one run	Condition of cells at conclusion of electrolysis. (Brown solution at start of electrolysis).
Cathode				
1	9.9-11.0	10.8	10.6	Color became lighter during electrolysis;tinge of yellow at conclusion;fine white suspension throughout solution.
2	7.4-10.2	10.1	9.5	Became turbid,but was clear light yellow brown at conclusion;slight ppt.
3	4.7-7.4	6.4	6.2	Color similar to cell 2 but slightly darker; some flocculent ppt.
4	3.5-3.9	3.7	3.7	Dark brown color;clear with several large masses dark brown ppt.
5	3.0-3.4	3.3	3.3	Clear yellow brown;flocculent ppt.
6	2.8-3.3	3.2	3.1	Color similar to cell 5;turbid with suspended particles;some ppt.
7	2.8-3.1	3.0	3.0	Light yellow brown;very turbid with considerable ppt.
8	2.1-2.5	2.4	2.4	Light yellow to nearly colorless;bottom covered with flocculent ppt;largest amount of ppt. in series of cells.
Anode				

Table II.- Growth of Lemna in sterile inorganic solutions supplemented with electrolyzed liver extract. Initial pH of cultures 4.8. Growth periods 20 and 30 days duration.

Culture Number	Supplement added to basic inorganic media. Initial addition	Second addition after 15 days .	Kx 1000 From Fig.1	Condition of plants. Standard of comparison - plants grown in inorganic media.
415	Inorganic media - (Standard).		70	
289	3 cc liver extract	3 cc liver extract	65	Small plants; normal color; medium length roots.
498	3/8 cc liver extract	3/8 cc liver extract	83	Slightly smaller than standard. Normal color; medium length roots.
172	3 cc No.1; pH 10.7*		70	Normal size; slightly lighter green; roots slightly shorter
499	3/8 cc No.1; pH 9.9	3/8 cc No.1; pH 10.7	85	Small plants; normal color; medium roots.
321	3 cc No.5; pH 3.2	3 cc No.6; pH 3.2	72	Small plants; light green color; medium roots.
406	3/4 cc No.5; pH 3.4	3/4 cc No.5; pH 3.3	79	Normal fronds; roots slightly shorter.
405	3/8 cc No.5; pH 3.4	3/8 cc No.5; pH 3.4	85	Normal fronds; slightly shorter roots.
502	3/16 cc No.5; pH 3.4	3/16 cc No.5; pH 3.4	93	Normal fronds; slightly shorter roots.
192	3 cc No.8; pH 2.1		62	Very small fronds; thick, short roots.
504	3/8 cc No.8; pH 2.6	3/8 cc No.8; pH 2.1	82	Normal; roots slightly shorter.

* Number of cell in electrolysis system and pH of contents; volume refers to the aliquot of cell contents added to the basal inorganic medium.

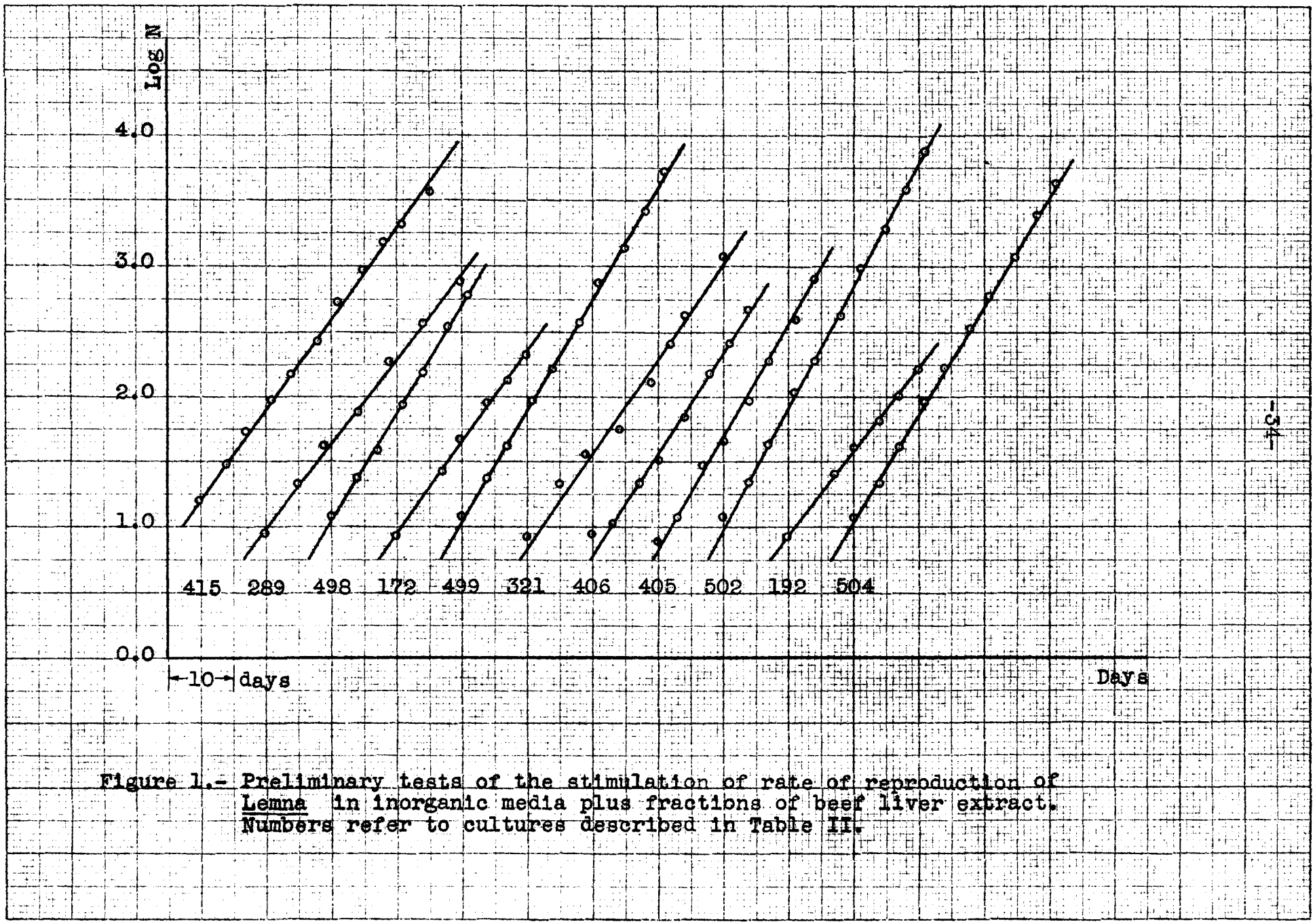

Figure 1.- Preliminary tests of the stimulation of rate of reproduction of Lemna in inorganic media plus fractions of beef liver extract. Numbers refer to cultures described in Table II.

Trends Indicated

The data presented in Table I include results from electrolyses carried out in the preliminary investigations in which extracts of dry beef liver were electrolyzed. A water solution of the material extracted by 80 percent methyl alcohol from five grams of dry beef liver was used in each run. The volume of the water solution was always the same, 833 cc., of which 800 cc. was electrolyzed; the electrolysis time was approximately 20 hours in each case.

During the electrolysis a pH gradient was established in the series of cells; there was a decrease in acidity from about pH 2.5 in cell 8 (anode), to about pH 10.5 in cell 1 (cathode). This is clearly indicated by the results recorded in Table I.

The variation in pH for the solution in the different cells is shown by the values given in Table I. The range of pH in each cell for the nine electrolyses is recorded in the first column under pH of cells. The greatest difference occurred in cells 2 and 3-- 7.4 to 10.2 for cell 2 and 4.7 to 7.4 for cell 3. The variation in pH for the other cells was confined within narrow limits; this was especially true for cells 4 to 8 inclusive, the final pH of the solution contained in each of the cells varying only from 0.3 to 0.5 of

a pH unit.

For the different electrolyses carried out, there was some overlapping of the range of final pH values, namely, cells 1 and 2 and 5, 6 and 7. The overlapping in cells 1 and 2 was small compared with the variation for the series of runs in the cells themselves. However, in cells 5, 6 and 7, the range of the group as a whole, 2.8 to 3.4 did not vary greatly from that of each individual cell; the average values and the values in the sample run put them still closer together, the range being 3.0 to 3.3. Cell 8 on the anode end of this group, stands alone with a range of 2.1 to 2.5; cell 4 on the cathode side, with a pH range of 3.5 to 3.9, while not so distinctly separated from the group 5, 6 and 7 as cell 8, nevertheless is in a position by itself in the pH gradient. The final pH of the solution in cell 3 was always removed from that of the cell on either side. The results of the nine electrolyses give it a pH range of from 4.7 to 7.4 and the average value pH, 6.4, places it approximately in the middle of a six unit gap between the averages for cells 2 and 4.

Williams and his co-workers (77), as previously stated, advanced the theory that the separation of the growth substance by electrolysis was due to the decrease in the ionization of the acid in solutions of a high hydrogen ion concentration. As the acidity increased there would be a con-

tinued decrease in the ionization of the acid and consequently less movement of the acid radical toward the anode in an electric field. On the basis of this theory it would seem possible, from the data recorded in Table I and previously discussed, to control easily an electrolysis so that the greater part of the acid would accumulate in cells 5,6,7 and 8; by means of a long electrolysis period the substance might be separated in cell 8.

The data recorded in Table II and the curves plotted in Figure I, are representative of the trends indicated by these preliminary experiments on the growth of Lemna in the inorganic medium containing prepared extracts of beef liver. Several interesting facts are brought out by these results.

The presence in the extract of substances which increased the rate of reproduction of Lemna is clearly demonstrated. The rate of growth for the standard, culture 415, as measured by K, was 70. The value for K was much greater for six of the supplemented cultures included in Table II, namely, cultures 498,499,406,408, 502 and 504. The solutions indicated represent cultures containing additions from the original water solution of the extract or portions of electrolyzed fractions from cells 1, 5 or 8; stimulation of the growth of Lemna was also obtained in nutrient solutions to which aliquots of the electrolyzed fractions from cells 4, 6 or 7 were added. No aliquot taken from cell 2 or cell 3

increased the growth of the plants; however, because of lack of space in the constant light and temperature apparatus, the contents of cell 2 were not fully investigated and small aliquots from it might have given results similar to those obtained with aliquots from cell 1 (culture 499). The stimulation of the growth of Lemna in solutions containing additions from cell 1, culture 499, was unexpected. Williams and others, (77), obtained only a slight stimulation of yeast growth from the basic electrolyzed fractions of liver extract. McBurney, et al, (48) did not mention the use of that fraction in their report of the stimulation of alfalfa seedlings by pantothenic acid.

The increase in the rate of reproduction of Lemna in the cultures containing the various forms of the extract of liver was always accompanied by changes in the appearance of the plants, especially noticeable in those cultures containing the largest volume of the supplement. The fronds became smaller, turned a lighter green color and lacked the waxy luster shown by plants grown in the standard solutions; the roots were shorter and in some instances decreased to mere stubs, e.g., culture 192. This effect of growth substances on roots has been reported by other investigators, Boysen-Jensen (7), found the root growth of beans was completely stopped in auxin solution; Kögl (41), from similar experiments, reported that roots of oat seedlings decreased

in length. Marmer (51), grew wheat seedlings in solutions containing beta-indolyl acetic acid and reported a decreased growth of the primary roots.

In addition, the data in Table II and the curves in Figure I, bring out one other point - an increased rate of reproduction accompanying a decrease in the volume of the various aliquots added to the nutrient solutions. This is shown very distinctly in Table II by cultures with additions from cell 5; Lemna grown in cultures 321, 406, 405, and 502, containing in that order 3, 3/4, 3/8, and 3/16 cc. portions from cell 5, had rates of reproduction expressed by K of 72, 79, 85, and 93. A similar relation is shown by the values for K for the other cultures recorded in Table II, which were supplemented with the original unelectrolyzed solution of the extract and from electrolyzed fractions from cells 1 and 8. The curves in Figure 1 present a graphic picture of this fact; the steepest curves are associated with the smaller volumes of added supplement. Other cultures were grown with larger or smaller aliquots from the cells between those recorded in Table II, but the group given are representative.

The rapid increase in the rate of reproduction of Lemna when grown in media supplemented with the small aliquots, both from the original unelectrolyzed solutions and from the electrolyzed fractions, indicates too high a con-

centration of the growth substance in the original solution. Furthermore, the marked stimulation from cells 4 to 8, inclusive, called for a longer period of electrolysis in order to concentrate the growth substance in fewer cells of the apparatus. With these points in mind a further investigation was carried out, the results of which are presented in the section entitled "Comparison of Effective Concentrations".

Series II : Test of chemical nature of growth-promoting material.

Preparation of media.

The object of this series was to determine if the stimulation in the growth of Lemna secured in Series I was due to inorganic or organic substances. During the preparation, information was secured on the amount of dry matter contained in the 80 percent methyl alcohol extract of dry beef liver. Data were also secured on the final distribution of material by the fractional electrolysis of the extract.

The procedure in the extraction of the liver and purification of the water solution of the extracted material was identical with that described under "Methods for Lemna", with the exception that a 5.28 gram lot of dry beef liver was used instead of a 5 gram portion, in order to secure a larger volume of the water solution of the extracted material. The concentration of extracted matter in the water solution pre-

pared for electrolysis was identical with those solutions prepared from the material extracted from 5 gram samples. A larger volume of solution was necessary so that aliquots of the solution electrolyzed could be reserved for further treatment with the electrolyzed fractions.

After an electrolysis period of approximately 20 hours, pH determinations were made on the contents of the cells. The material in the cells was well mixed and two aliquots were removed from each cell to tared porcelain crucibles. These crucibles, together with those containing the portions of unelectrolyzed solution, were placed in a sand bath and the contents evaporated to dryness; after removal from the sand bath, the crucibles containing the dry residues were heated to constant weight at 105° C., and one of each pair was ignited at dull red heat. Following ignition, the crucibles were again weighed. Ten cc. of 1:3 hydrochloric acid were added to each ashed residue, the crucibles placed in the sand bath and the contents evaporated to dryness; then ten cc. of water were added and the contents of the crucibles again evaporated to dryness. A blank, containing the same volume of HCl used for the samples of ash, was evaporated with the samples. Following treatment, the crucible was washed with water and the washings prepared in the same manner as the water solutions of the treated ash.

The sample of treated ash and the blank were taken up in water, filtered and the filtrates made up to 100 cc. The dry matter duplicates of the ignited material were also taken up in water and made up to 100 cc. Some of the dry matter was insoluble in water; this was broken into fine particles and left in the 100 cc. of solution. The sediment was mixed with the liquid before removing aliquots to supplement Lemna cultures. The measured portions were added to the inorganic media and the completed duplicated cultures prepared and inoculated with Lemna as outlined in "Methods for Lemna". As in Series I, it was necessary to use material from two separate lots of liver in order to secure sufficient cultures to continue the experiment for at least a month.

Trends Indicated.

The data recorded in Table III were secured from the determination of dry matter, in duplicate portions, from the electrolysis of two lots of dry beef liver. The results from the ignition of the residue of one of the duplicates of each determination of dry matter are included in the table.

The pH gradients shown by the two values recorded in

Table III.- The determination of dry matter and ash in water solution(unelectrolyzed and electrolyzed fractions) from 80 per cent methyl alcohol extract of dry beef liver.

Source of Sample	pH Samples Two electrolyses (Glass electrode).	Dry matter** Average value four aliquots mg/cc.	Ignited dry matter (dull red heat) from one 40 cc. aliquot for each sample. Weight dry matter	Weight ash mg.	Percent ash in dry matter.
Original solution*	4.9, 4.9	1.0	41.3	3.4	8.2
Cathode					
Cell 1	10.8, 11.0	1.3	53.8	24.2	44.9
Cell 2	10.1, 9.8	0.8	35.3	5.6	15.9
Cell 3	6.8, 6.4	0.8	29.9	Trace	----
Cell 4	3.7, 3.7	0.9	37.8	Trace	----
Cell 5	3.3, 3.3	1.0	43.1	1.8	4.2
Cell 6	3.2, 3.2	1.1	47.7	3.0	6.3
Cell 7	3.0, 3.0	1.25	50.6	2.9	5.8
Cell 8	2.4, 2.4	1.4	55.9	5.5	9.8
Anode					

* Unelectrolyzed water solution of 80 percent methyl alcohol extract of dry beef liver.

** Average value of dry matter determinations on four aliquots (two 25 cc and two 40 cc) taken from electrolyses of water solutions of 80 percent methyl alcohol extract of two 5.28 gram samples of dry beef liver.

the second column of Table III are similar to those given in Table I in the preceeding section of this thesis; neither the trend of the pH gradient nor the distinct differences in pH from cell to cell appear to be correlated with the distribution of dry matter. The dry matter values are recorded in the third column of Table III as milligrams per cc. of the solution evaporated; the values given are the average of four determinations. The largest amount of material was found in the two extremes of reaction, cell 1, cathode, most alkaline and cell 8, anode, most acid. From cell 1 to cell 2 there was an abrupt decrease in dry matter, 1.3 mg. per cc. for cell 1, compared to 0.8 mg. per cc. for cell 2; the accompanying change in pH was from 10.8 or 11.0 for cell 1 to 10.1 or 9.8 for cell 2. From cell 2 to cell 3, however, there was a change in final pH of approximately 3 pH units, while there was no change in the amount of dry matter. Likewise, the large increase in acidity from cell 3 to cell 4, was not accompanied by a corresponding difference in dry matter. From cell 4 to cell 7 a gradual increase in dry matter was recorded with increasing acidity, and the sharp increase in acidity from cell 7 to cell 8 was accompanied by a slightly greater increase in dry matter between these cells as compared to the acidic cells with nearly equal pH differences.

The last three columns of Table III contain results

from the ignition, at dull red heat, of one sample of the dry matter from the various evaporated aliquots. An exceptionally high value is recorded for the ash remaining after ignition of the material obtained from cell 1; the residue not volatilized at dull red heat was 44.9 percent of the dry matter. The ignited residue had a black appearance and it is doubtful if all the organic substances were ignited at the temperature used. This appears probable from the average value (not recorded in Table III) for weights of ash for the series which is approximately six and is nearly twice the 3.4 mg. value given for the unelectrolyzed solution. A high percentage, 15.9 of ash in the dry matter is also shown for cell 2. Cells 3 and 4 contained only a trace of ash, while the trend of the results obtained for the ash of the residues from cells 5 to 8 is similar to the changes in dry matter and final pH values.

The values recorded cannot be considered as final since they are the results of but one determination; yet the results obtained with Lemna in cultures containing additions of the prepared materials, clearly show that the growth-promoting substance present in any of the fractions, was destroyed by the ignition at dull red heat.

Table IV presents data on the rate of reproduction of Lemna in solutions supplemented with portions of dry matter compared with the rate of reproduction in solutions supplemented with a 1:3 HCl extract of the samples ignited at dull

red heat. The values given for the rate of reproduction, K, were obtained graphically from the curves plotted in Figure II. In this figure, curve 447 represents the standard. No data for this culture are included in Table IV.

The rate of growth, K, for the standard solution in Series II was 70; compared with it, the rate of reproduction was greater for all cultures supplemented with the various forms of dry matter recorded in Table IV, while none of the values for K was greater than 70 for the cultures containing additions of the 1:3 HCl extract. This comparison is shown by the curves plotted in Figure 2. The first curve, 447, represents the standard. The next five pairs of curves are for the five sources of material tabulated in Table IV; the steeper curve of each pair represents the rate of growth of Lemna in the cultures supplemented with dry matter and the less inclined curve shows the similar value for the cultures containing additions of 1:3 HCl extract. The cultures in all instances contained equivalent amounts of material.

The comparison of the rate of reproduction of the cultures supplemented with the two kinds of material, clearly shows that ignition of the dry matter at dull red heat destroys the growth-promoting substances. This result was obtained also for cultures supplemented with materials from cells 4, 6 and 7; which cultures were not included in the

Table IV.- Comparison of the growth of Lemna in inorganic solutions containing additions of material extracted by 80 percent methyl alcohol from dry beef liver. Dry matter obtained by evaporation to dryness of water solutions of the prepared material. Ash of dry matter (ignited to dull red heat) added as filtered water solution of 1:3 HCl extract. Added materials equivalent to 3 cc. of original solutions.

Original material from two electrolyses		Water solution of dry matter			Water solution of 1:3 HCl extract of ash of dry matter		
Source	Average pH (Glass electrode).	Culture Number.	Amount dry matter added** mg.	Kx 1000 From Fig. 2	Culture Number.	Amount ash added as HCl extract *** mg.	Kx 1000 From Fig.2
Unelectrolyzed solution.	4.9	444	3.0	78	459	0.255	59
Cathode							
Cell 1	10.5	433*	3.9	82	448	1.815	66
Cell 3	6.4	434	2.4	74	450	Trace	62
Cell 5	3.3	440	3.0	82	455	0.135	62
Cell 8	2.4	443	4.2	86	458	0.412	63
Anode							

* Non-sterile; contaminated.

** Average value of dry matter determinations on four aliquots (two 25 cc. and two 40 cc.) taken from electrolyses of water solutions of 80 percent methyl alcohol extract of two 5.28 gram samples of dry beef liver.

*** Results from ignition (dull red heat) of dry matter from 40 cc. aliquot.

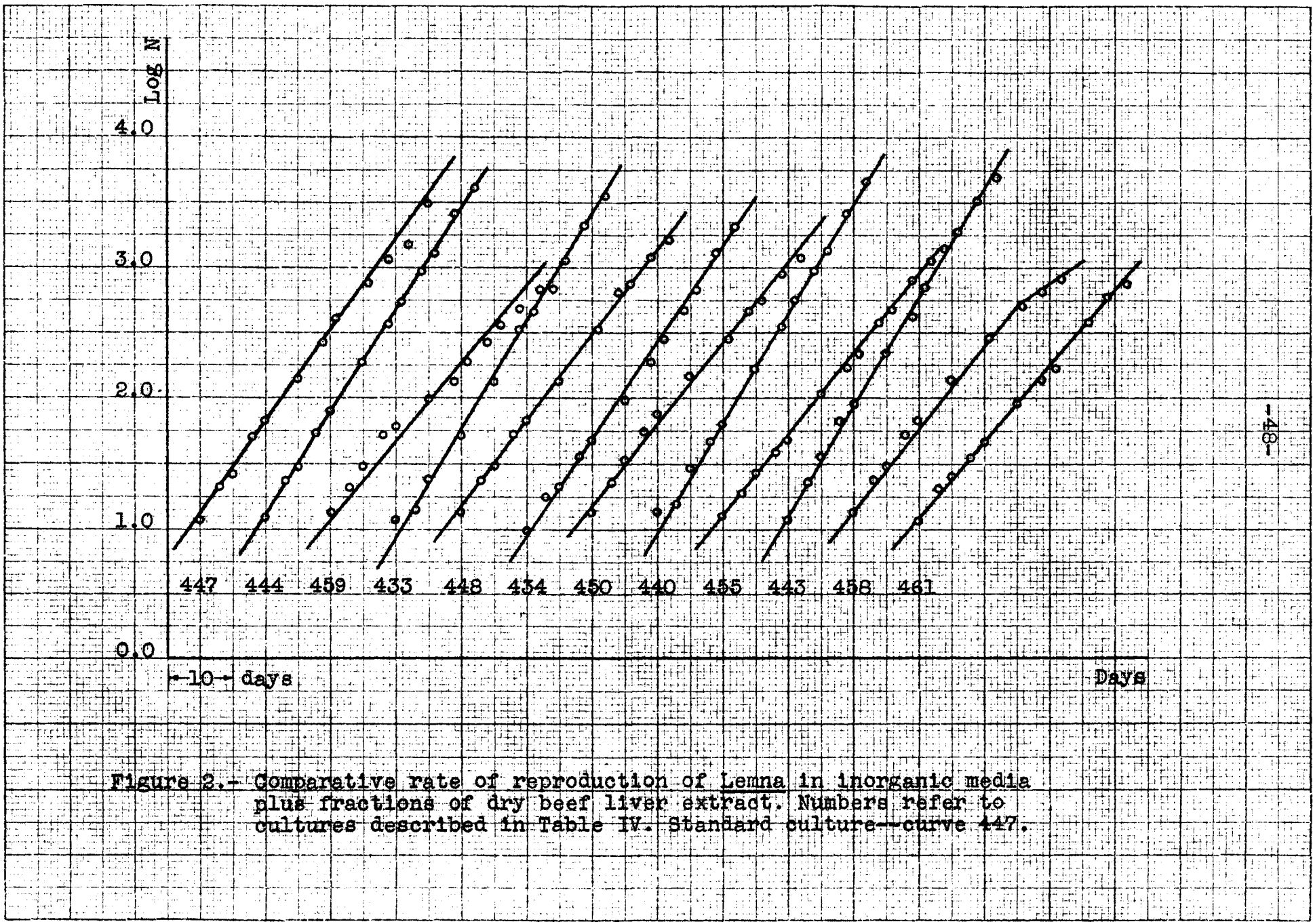

Figure 2.- Comparative rate of reproduction of Lemna in inorganic media plus fractions of dry beef liver extract. Numbers refer to cultures described in Table IV. Standard culture--curve 447.

data tabulated in Table IV. Neither the dry matter of cell 2 nor an extract of its ash was added to cultures for Lemna.

The rate of reproduction, K , for the 1:3 HCl extract cultures, was consistently less than the similar value for the standard culture (culture 447, K = 70). This was probably due to failure to adjust the reaction to 4.8 for the nutrient solutions after the 1:3 HCl extract was added. The last curve plotted in Figure 2 is for culture 461 which contained an equivalent aliquot from the evaporated blank portion of 1:3 HCl. The rate of reproduction, K, for this culture, was 58; this value seems to attribute the slow rate of growth of Lemna to the acid in the cultures containing ash extract. Also, K=66 for culture 448, which contained the 1:3 HCl extract of ash from cell 1 (cathode cell and most alkaline of the series); this was the highest value for K in the series of ash extract cultures. The ash in cell 1 would contain alkaline residues which would help to neutralize any excess HCl not removed by evaporation.

The striking difference in growth rates for the two forms of supplement was accompanied by a difference in the condition of the plants at the end of the experiment. The Lemna grown in ash extract cultures 448, 450, and 455, were very similar in size, color and general appearance to the standard (culture 447). Similar results were obtained for ash extract cultures not included in the selected data given

in Table IV, namely, cultures supplemented from cells 4, 6 and 6. In cultures 458 and 459, supplemented with extract of ash from unelectrolyzed solution and from cell 8, respectively, the plants were smaller than the standard and had a light green color. The groups of fronds were irregular in number and loose roots were usually present in the cultures. The points on the curves for these two cultures in Figure 2, also show irregularities in the rate of growth.

The appearance of the plants in the dry matter supplemented cultures, with the exception of size, was very similar. Compared with the standard, the plants were a lighter green and showed an absence of the waxy luster which gave the Lemna grown in inorganic medium a plump, glossy appearance. Plants grown in the supplemented cultures had ridged, hard appearing fronds. The Lemna in cultures supplemented from cells 1 to 7, inclusive, (cultures containing additions from cells 4, 6 and 7, not included in selected data for Table IV), were only slightly smaller than the standard; the roots in all cases were somewhat shorter compared with the plants in the inorganic media. Plants in culture 444, unelectrolyzed material, and culture 443, material from cell 8, were considerably smaller than the standard and also had short roots. Culture 433, containing dry matter from cell 1, became contaminated toward the end of the growth period. A culture containing supplement from the same source was carried through with a series of cultures

in another experiment; the rate of growth of Lemna in the second culture was approximately the same as for plants grown in culture 433.

The trends indicated by the results from the growth of Lemna in the dry matter supplemented cultures of this series, (Series II), were in general the same as those indicated for the preceding series. Two differences, however, are notable. The Lemna in culture 443, supplemented with dry matter from cell 8, had a value for K of 85 as compared to K = 62 for culture 192. Series I, Table II, culture 192, contained a 3 cc. aliquot of the contents of cell 8, while culture 443 contained dry matter equivalent to that volume from cell 8. Evidently the evaporation of the original solution from this cell either had removed substances toxic to Lemna, or some of the growth substance was destroyed in the process, leaving a more optimum concentration in the dry matter for the growth of Lemna.

The second point of difference between Series I and Series II is the slight stimulation of the growth of Lemna in culture 434, supplemented from cell 3; no increased rate of reproduction of Lemna in cultures containing additions of the solution from this cell was obtained in the first preliminary series. Furthermore, Lemna with a rate of reproduction K = 80 were grown in a culture containing twice the amount of supplement from cell 3 as was added to culture 434, (data for this

culture are not tabulated in Table IV). The stimulation of Lemna in cultures containing additions from cell 3, brings out again a fact indicated by the results from Series I, i.e., too high an initial concentration of growth substance in the pre-electrolyzed solution, which prevented a complete separation into a few cells of the electrolysis apparatus. However, no cause is known for the increased growth of Lemna in the cultures of Series II supplemented from cell 3, where no increase of growth rate was evidenced in Series I, although the possible removal of toxic substances in the preparation of the dry matter might be an explanation.

The growth of Lemna in the supplemented cultures used in the two preliminary series indicates the presence of an organic growth-promoting material in the 80 percent methyl alcohol extract of beef liver which will stimulate green plants. The effective concentrations suggested for the optimum growth of Lemna under the growth conditions of the experiments, were used in a third and concluding series of cultures.

Series III. Comparison of Effective Concentrations.

Preparation of media.

In the preliminary experiments, the residue obtained from extraction of dry beef liver was taken up in water for further concentration by electrolysis. The volume of the

water solution was such that one cc. was equivalent to 6 mg. of the dry beef liver. Aliquots from this and the electrolyzed fractions were added to inorganic media to be used for the growth of Lemna and the reproduction of the plants was found to be faster in those cultures containing as small a portion as 3/8 cc.; even smaller aliquots of the electrolyzed fractions gave still more rapid growth. The amount of dry beef liver extracted was therefore reduced from 5 gm. to 1.2 gm. for the series of cultures designed to compare the effective concentrations of growth-promoting material. One cc. of the water solution which was electrolyzed was equivalent to approximately 1.5 mg. dry beef liver, as compared with the value of 6 mg. for the first two series. Except for the decrease in amount of dry beef liver, the procedure in preparing the 80 percent methyl alcohol extract was identical with that outlined under "Methods for Lemna ".

Results from the preliminary experiments also indicated that a longer period of electrolysis might yield a more effective separation of the growth-promoting material. Accordingly, the time of electrolysis was increased to 58 hours, nearly three times the length of time used in the preliminary electrolyses. The conductance of the more dilute solution was much less than that of the more concentrated water solutions of the extracted material. The initial

current strength was 1.0 milliampere which gradually decreased during the interval of electrolysis to approximately 0.1 milliampere; an initial voltage of 1950 volts increased gradually to 2000 volts at the termination of the operation. At the end of the period of electrolysis, the condition of the contents of the separate cells of the apparatus was similar to that described in Table I for the preparation of the more concentrated solutions.

After the electrolysis, the final pH of the contents of the cells was measured with a glass electrode. Aliquots were then removed and added to inorganic media to prepare cultures for Lemna. The entire preparation of the cultures was identical with the procedure described under "Methods for Lemna", except that in this experiment sufficient material was obtained from one lot of dry beef liver to prepare the necessary number of cultures to complete the entire experiment - the previous series had required the extraction and electrolysis of two separate portions. The periodic transfer of Lemna was made at five day intervals; this method was identical with that used in previous experiments.

The results of all cultures in the series, except the standard, are given in Table V. The condition of the plants in the standard was normal and was used as the basis for comparison. The rate of reproduction, K, was obtained graphically from the curves plotted in Figure 3. The first

curve, number 665, represents the standard.

Results.

The tabulated data in Table V include an expression of the pH gradient established at the conclusion of the electrolysis. The final pH values for all the cells, except cell 2, are recorded in the third column. Compared to previous electrolyses, the longer period used and the decrease in concentration of the original water solution, produced some changes in the variation of the final pH values from cell to cell in the series. The pH of cell 1, cathode cell, was 10.6, which was very similar to previous recorded values. The final pH for cell 2 was 8.2 ; this is a more distinct variation from cell 1 than in former electrolyses. Continuing toward the anode, the variations between cells show a gradual decrease until between cells 7 and 8 there is a more distinct difference in the final pH. In the pH gradient, cells 6 and 7 fall closely together, while in the preceding electrolyses cell 5 was also closely associated in final pH value with cells 6 and 7.

The largest amount of precipitate was located in the more acid cells. This result was obtained also in the preliminary electrolyses of more concentrated solutions. This pointed to a distribution of dry matter in the cells similar

Table V.- Influence of the effective concentrations of various fractions of extract of dry beef liver on the rate of reproduction of *Lemna*.

Culture Number.	Material added to inorganic media. Source	pH Glass electrode.	Volume of added aliquot cc.	Kx1000 From Fig. 3.	Condition of plants. Standard of comparison--*Lemna* grown in inorganic media. Kx1000 = 72.
652	Unelectrolyzed solution.	4.9	3.0	90	Small plants; normal color; short roots.
653	Cathode Cell 1	10.6	3.0	85	Very small plants;normal color; very short roots.
654	Cell 3	6.1	3.0	80	Slightly reduced size;normal color; slightly shorter roots.
655	Cell 4	4.9	3.0	70	Larger, plump plants; better appearance;long, heavy roots.
656	Cell 5	4.1	3.0	84	Normal plants; fewer roots.
657	Cell 6	3.7	1.5	87	Slightly smaller,normal green; shorter and fewer roots.
658	Cell 6	3.7	3.0	91	Identical with plants in 657.
659	Cell 7	3.5	1.5	91	Normal size;lighter green; fewer and shorter roots.
660	Cell 7	3.5	3.0	90	Slightly reduced size;lighter green;fewer and shorter roots.
661	Cell 8	2.8	1.5	90	Small, normal green plants; short roots.
662	Cell 8 Anode	2.8	3.0	96	Identical with plants in 661.

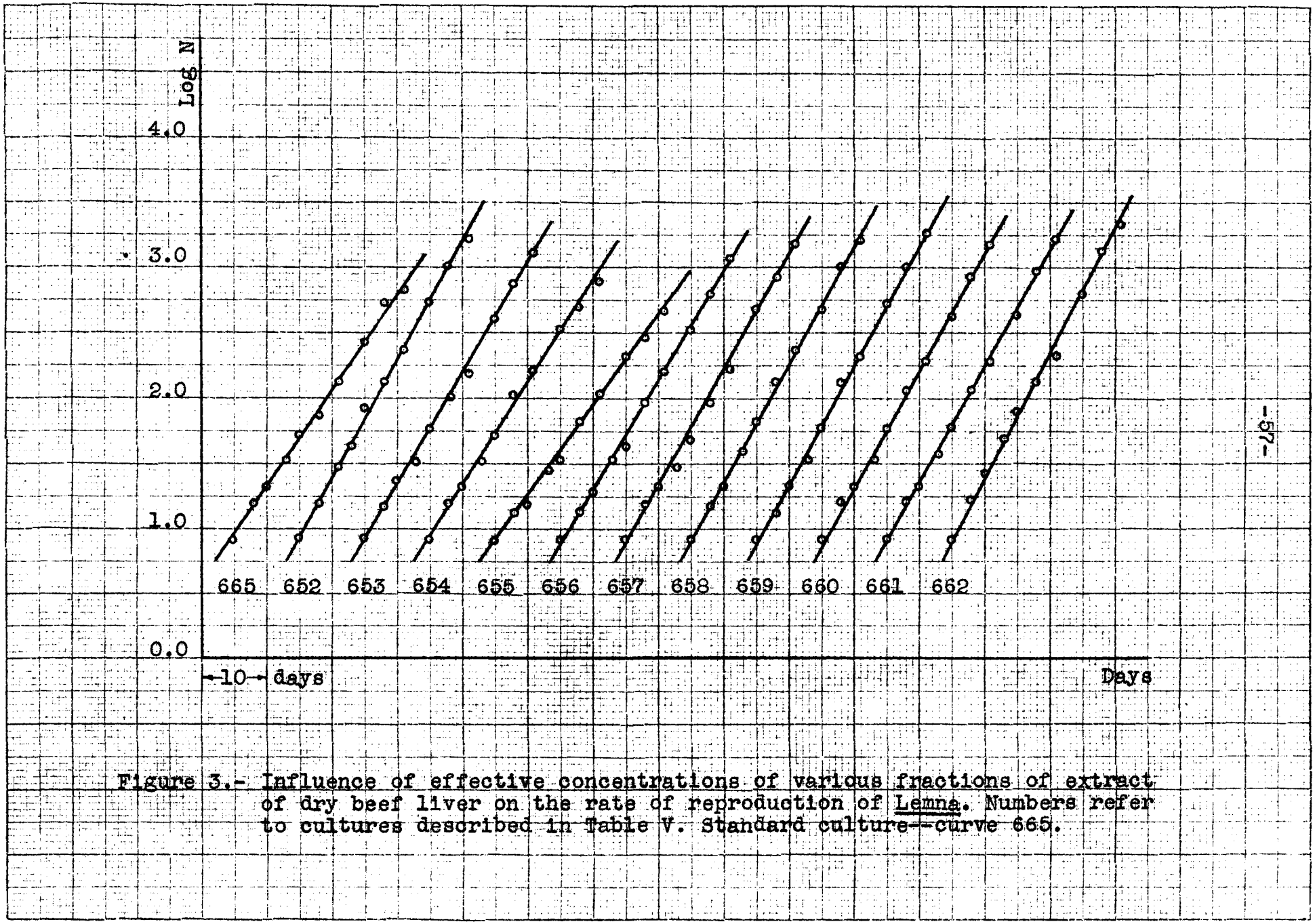

Figure 3.- Influence of effective concentrations of various fractions of extract of dry beef liver on the rate of reproduction of Lemna. Numbers refer to cultures described in Table V. Standard culture--curve 665.

to that found in the previous separation for Series I and II, and no further determinations were made of the material.

The aliquots of supplement added to the inorganic medium to prepare cultures for Lemna are given in the fourth column of Table V and the values for the rate of reproduction, K, in the fifth column.

The Lemna in supplemented cultures, with one exception, had values for K from 80 to 96, which were consistently greater than the rate of reproduction K=72 for the plants grown in the standard culture; for the exception noted, culture 655, containing a 3 cc. aliquot from cell 4, K=70. The values for K were obtained graphically from the curves plotted in Figure 3, curve 665 representing the standard. The steeper slope for all the curves (except 655) shows clearly the increased rate of growth of Lemna in the supplemented cultures; curve 655 is similar to the curve for the standard.

On the basis of the rate of reproduction, K, for the supplemented cultures, the growth substance for Lemna was removed entirely from cell 4 only, and a concentration of the growth substance greater than that of the unelectrolyzed solution was obtained in cell 8 only. This is evident from the fact that plants in 652, containing 3 cc. of the unelectrolyzed solution, give a value K=90, and plants in 662 containing 3 cc. from cell 8, K=96. The 96 is the only significantly larger value than the 90 of the unelectrolyzed;

all other cultures in this series have values either equivalent or smaller. For aliquots of 1.5 cc. from the cells, the comparison of the rates of reproduction indicates also that the growth substance was concentrated in the anode cells.

The values for K show a movement of growth-promoting material for Lemna toward both anode and cathode, suggesting the separation and concentration of two different substances by the electrolysis. According to the rate of reproduction of the plants, cell 4 was the division point in the separation of the material. The values for K also show that either the concentration of the substance moving toward the anode was much greater than the substance moving toward the cathode, or that the potency of the anode fraction was greater. The greatest concentration of each was separated in the electrode cells. Although aliquots from cell 4 did not stimulate the rate of reproduction of the plants, something beneficial was added from this source as the appearance of the plants when compared with those in the standard inorganic medium was distinctly improved.

The condition of the plants at the end of the growth period is given in the final column of Table V. Culture 655, supplemented from cell 4, was the only culture to contain plants which were superior to those in the standard. As a general rule, reduced size of fronds and shorter and fewer roots were associated with an increased rate of growth .

Fronds were also a lighter green in color compared with the normal green of the standard. A marked decrease in size of the fronds is recorded for cultures 661 and 662, supplemented from cell 8 (anode and most acid), and for the Lemna in culture 652 containing additions of unelectrolyzed solution; very small plants were grown in culture 653, supplemented from cell 1 (cathode and most alkaline). In each instance, the marked decrease in frond size was accompanied by a similar decrease in length of roots.

Discussion.

The results on the growth of Lemna in the three series of cultures show that some stimulating substance or substances for Lemna are extracted from dry beef liver by 80 percent methyl alcohol. The indications that too large a concentration of material was used in the two preliminary series were substantiated by results secured from Series III; but the comparison of the results of unelectrolyzed and electrolyzed material for Series III suggests that a still lower concentration might be desirable. These facts are shown by the rate of reproduction, K, for Lemna in the supplemented cultures, and by the condition and appearance of the plants at the end of the growth period.

An increased growth rate for Lemna was obtained in

Series III for supplement from all cells used, except cell 4. The values for K show the greatest concentration of growth substance in cells 6, 7 and 8, and the concentration of growth-promoting material no greater in cells 6 and 7 than in the unelectrolyzed solution - equivalent aliquots from these last three sources gave nearly identical stimulation to Lemna. A similar aliquot from cell 8 was distinctly superior to these sources in ability to increase the rate of reproduction.

Although the stimulation of the growth of Lemna by the acidic electrolyzed fractions of dry beef liver extract checks the results reported for yeast by Williams and his co-workers (77), the stimulation obtained for Lemna in these experiments from the neutral and alkaline fractions was not as marked for the yeast. McBurney, Bollen and Williams (48), used only the concentrate obtained from the acidic fractions in the experiments which showed stimulation of alfalfa seedlings in sterile cultures. That there was a stimulation of Lemna by the neutral and alkaline fractions was indicated in Series II and clearly shown by the results of Series III.

Some movement of the growth-promoting material toward the cathode in an electric field is indicated by K= 85 for Lemna in culture 653, supplemented from cell 1, as compared with K = 80 for culture 654, supplemented from cell 3. Cell 2 was not used to prepare cultures for Lemna and no data are available for it. The concentration of growth material toward

the electrodes by the electrolysis was also shown by the condition of the plants.

The reduction in size of the stimulated plants compared with Lemna grown in inorganic medium suggests that an increase in the rate of reproduction was maintained by sacrificing development of normal frond size. Supplemented plants which reproduced at nearly the same rate as the standard showed no reduction in the size of fronds.

A marked inhibition of root growth, both in number and length, seemed always to accompany optimum to high concentrations of the growth material extracted from the beef liver. This relation indicates that the growth substances which stimulate the rate of reproduction of fronds produce the opposite effect on the roots; but the decreased root length of stimulated Lemna correlates with the diminution in size of the fronds. Kögl, Haagen-Smit and Erxleben (41) found that auxin a, a growth promoter for oat coleoptiles, also exerts an inhibitory effect on the roots of oat seedlings grown in nutrient solutions containing the substance.

A further criterion of plant condition, the color of the fronds, was very similar in all cultures, with the exception of 659 and 660 (cell 7) in which the plants were lighter green than the standard. A lighter green color was also reported by McBurney, Bollen and Williams (48) for alfalfa seedlings when stimulated by pantothenic acid in

sterile cultures. The waxy luster, so prominent in plants of the standard, was present in all cultures and especially marked in the Lemna in culture 655, cell 4.

Summary and Conclusions on Pantothenic Acid.

Growth-promoting material for Lemna was prepared by extracting dry beef liver with 80 percent methyl alcohol; a water solution of this extract was concentrated by fractional electrolysis. Aliquots from the unelectrolyzed solution and from the electrolyzed fraction were added to inorganic media in which Lemna were grown under sterile conditions.

Under the growth conditions of experiments carried out, the rate of reproduction of Lemna was increased by the material extracted from the dry beef liver by 80 percent methyl alcohol. Ignition of residues obtained by evaporation of the prepared extract destroyed the growth-promoting property.

The extracted growth material can be concentrated by fractional electrolysis. The substance, or substances causing the greatest increase of the rate of reproduction of Lemna was concentrated toward the anode. This result checks the stimulation of yeast reported by Williams for a similar preparation, and also the stimulation of alfalfa seedlings in sterile cultures by pantothenic acid recorded by McBurney, Bollen and Williams.

Further, a substance or substances, which stimulated the rate of reproduction of the plants moved toward the cathode and tended to be concentrated in the cathode cell. Williams did not find that yeast was stimulated by additions from the cathode end to any marked degree, but there is a definite increase in his yeast reproduction with the larger of his two additions of the extract. McBurney, Bollen and Williams used only a concentrate from the acidic fraction (anode) for the stimulation of the alfalfa seedlings.

Increased rate of reproduction of Lemna was accompanied by a decreased production of chlorophyll, a reduction in size of the fronds and inhibition of root growth. McBurney and his co-workers also reported a loss of chlorophyll for alfalfa seedlings. Pantothenic acid thus stimulates the reproductive function in the Lemna as it did in the yeast, but in the case of the green plant it seems to be at the expense of normal root and leaf development.

AUXINS.

Investigations on the production in the tips of coleoptiles of grasses of the growth-promoting substances called auxins were outlined in the section entitled "History".

Numerous investigations of the physiological action of the auxins on plants led to the discovery of a variety of rich sources; oils of seeds, seedlings of plants, buds of trees, yeast, mold cultures and human urine are materials in which the auxins are plentiful. To determine the effect of these substances upon the rate of reproduction of Lemna, the roots and shoots of freshly germinated corn were selected as a source of auxins. Dollfus (22) and Kornmann (43) found corn seedlings to be rich in auxins, and Leonian (45) demonstrated the presence of a growth substance, similar to auxins, in the roots of germinated corn.

Methods for Lemna.

Yellow dent corn was placed in beakers, covered with tap water and allowed to soak overnight. The corn was then removed and put on moist filter paper in large crystallizing dishes to germinate. The dishes were fitted with covers to allow circulation of air and the temperature maintained at 37-38° C. Water was added to the dishes several times during

the germination period to replace loss by evaporation. At the end of three days the sprouts of corn had reached a length of 1/4 to 1 inch and the roots 1 to 2 inches; the kernels were then separated and the sprouts and roots extracted separately with 95 percent ethyl alcohol.

The severed corn shoots were divided into groups of 25; each group was placed in a mortar with 10 cc. of the alcohol and crushed with a pestle. After all the material was broken up, the alcohol was decanted and the extraction procedure repeated twice. The combined alcohol extract from the several lots of shoots was filtered free of suspended material and the filtrate evaporated to dryness on a water bath. The residue was taken up in water and the resulting solution filtered; the filtrate was combined with 50 cc. dilute phosphoric acid (0.0145 g. per liter) and made up to a known volume for further concentration by fractional electrolysis. The acid was added to increase the conductance, as that of the original solution was too low to carry the current. This method was used by Williams (77), in the electrolysis of plant extracts which had a very low conductance.

The procedures in extraction and electrolysis of the extracts were identical for the shoots and roots. The electrolysis apparatus was that previously described under "Methods for Lemna" for pantothenic acid.

Before placing the water solution in the electrolysis apparatus, aliquots were removed for supplementing inorganic media with unelectrolyzed solution. The electrolysis was continued for 48 hours; an initial current strength of 1.5 milliamperes decreased to 0.3 milliamperes at the conclusion of the separation, and during the same period the voltage remained at approximately 1950 volts.

At the termination of the electrolysis the pH of the contents of the cells was determined with the glass electrode. The contents of the cells was then well mixed and aliquots added to basal inorganic media to prepare cultures for Lemna. The preparation of the supplemented solutions, inoculation of the prepared media with plants, and the growth conditions for the cultures, were identical with those described in "Methods for Lemna" under pantothenic acid.

Experimental.

Preliminary experiments to determine the effective concentrations were carried out with the prepared 95 percent alcohol extract of shoots and roots. Results from these investigations indicated that a water solution which contained the extract of 200 to 300 shoots or roots in the volume required for electrolysis might yield fractions containing an optimum concentration of growth-promoting mater-

ial for Lemna.

Media Supplemented with Material from Corn Shoots.

The data given in Table VI are for Lemna grown in a series of cultures supplemented with aliquots of unelectrolyzed solution and electrolyzed fractions of a water solution of extract of corn shoots. The rate of reproduction for the plants was obtained graphically from the curves plotted in Figure 4. Curve 381 is for the Lemna grown in the standard inorganic medium; data for this culture are not recorded in Table VI.

Results.

The pH gradient established at the conclusion of the electrolysis of the extract of corn shoots is given in the third column of Table VI. The contents of cell 1 (cathode) has the only alkaline reaction in the series. A pH of 5.2 was obtained in cell 2, a large increase in acidity compared with cell 1. The pH values for the remaining cells of the series were closely grouped, ranging from 4.0 for cell 3 to 2.5 for cell 8 (anode).

There was very little change in the appearance of the solution during the electrolysis. The small amounts of

Table VI.- Influence of effective concentrations of various fractions of extract of corn shoots on the rate of reproduction of Lemna.

Culture Number.	Description of supplement* Source	pH Glass electrode	Kx 1000 Fig. 4	Condition of plants-- standard of comparison--Lemna grown in inorganic media. Kx1000 = 72.
707	Unelectrolyzed solution	-----	94	Lighter green color; small fronds; roots medium length.
	Cathode			
711	Cell 1	9.5	95	Slightly smaller fronds; lighter green color; medium length roots.
714	Cell 2	5.2	92	Lighter green, slightly smaller fronds; slightly shorter roots.
715	Cell 3	4.0	95	Lighter green color; small fronds; medium length roots.
716	Cell 4	3.5	90	Identical with 715.
719	Cell 5	3.3	95	" " "
720	Cell 6	3.2	94	" " "
721	Cell 7	3.0	95	" " "
722	Cell 8 Anode	2.5	92	Small dark green fronds; waxy luster; medium length roots.

* 3 cc. aliquot from each source was added to basal inorganic media. 3 cc. unelectrolyzed solution equivalent to extract of one shoot.

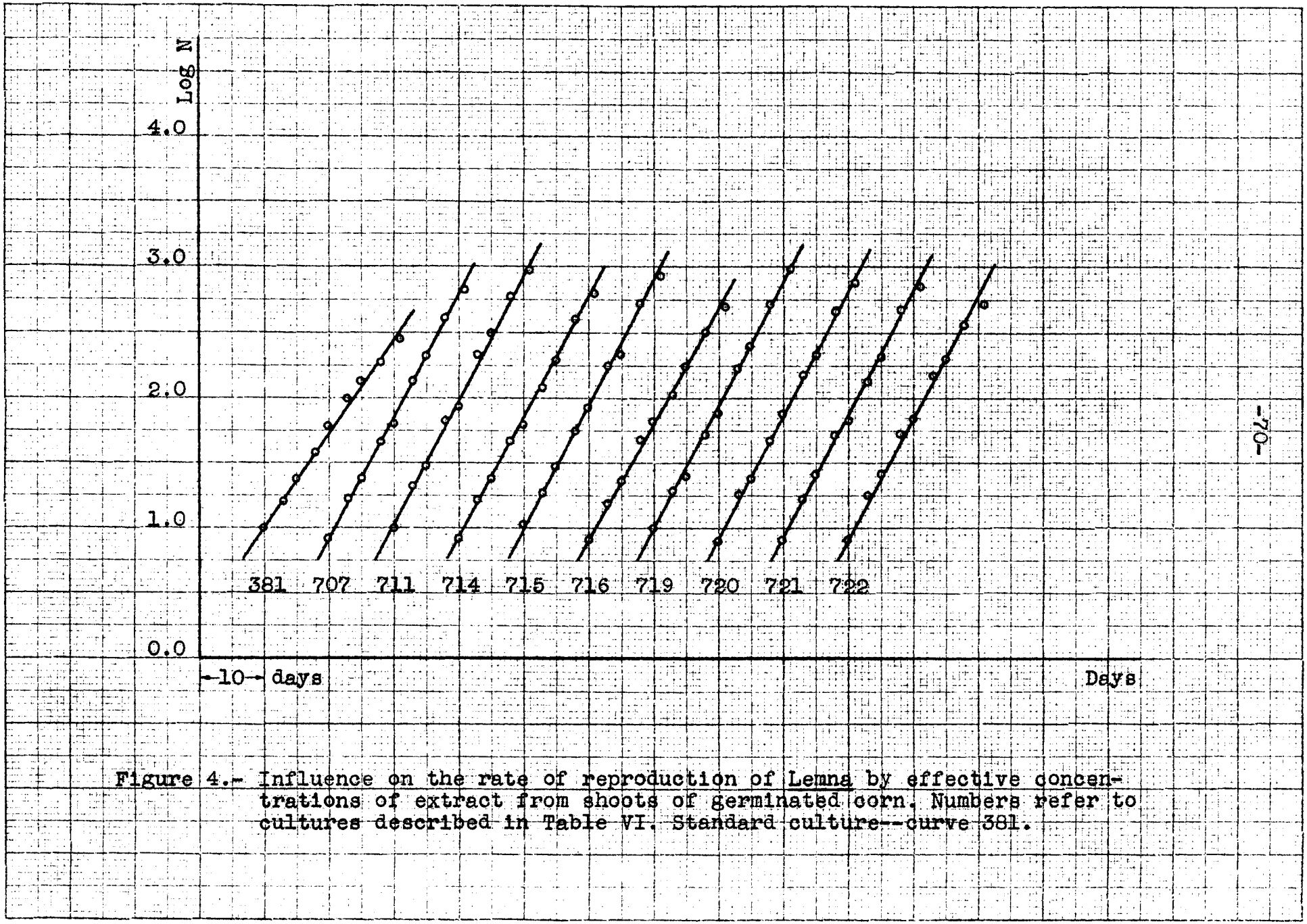

Figure 4.- Influence on the rate of reproduction of Lemna by effective concentrations of extract from shoots of germinated corn. Numbers refer to cultures described in Table VI. Standard culture--curve 381.

precipitate which formed collected in the electrode cells.

The rate of reproduction, K, of the Lemna grown in the supplemented cultures is recorded in the fourth column of Table VI and shown by the curves plotted in Figure 4. Lemna in supplemented cultures had values for K from 90 to 95 - a distinctly superior rate of growth compared with K = 72 for the plants of the standard culture.

The close grouping of the values for K of plants in the supplemented cultures indicates very little separation or concentration of the growth-promoting material in any particular cell or cells. This is shown by K = 94 for culture 707 containing unelectrolyzed solution, compared with values of 90 to 95 for the cultures containing electrolyzed fractions. The slight variations in the rate of reproduction are irregular and the faster rates were not obtained from any centralized point in the series of cells. This fact is brought out by the curves plotted in Figure 4; the differences in slopes of the curves for the supplemented cultures are very small.

The slight variations in the rate of reproduction of the Lemna in supplemented cultures were accompanied by a similarity in appearance among themselves, but a difference compared with the Lemna of the standard. The fronds of the plants in the supplemented cultures were reduced in size and had a lighter green color; the waxy luster of the standard

was absent. One exception was noted, culture 722, supplemented from cell 8, in which the fronds, although smaller, were similar to those of the standard in color and appearance.

A reduction in the length of roots is recorded in Table VI for the supplemented plants. The extent of the decrease in root length was approximately the same in all of the cultures containing additions of the prepared extract.

Media Supplemented with Material from Corn Roots.

Material was prepared for this series of cultures by the electrolysis of a water solution of the alcohol extract of approximately 225 roots. The data given in Table VII were secured from Lemna grown in cultures supplemented with the unelectrolyzed and electrolyzed fractions of the solution. The values for the rate of reproduction, K, recorded in the table, were obtained graphically from the curves plotted in Figure 5. The first curve in Figure 5, curve 381, is for Lemna grown in inorganic media. Data on this culture are not given in Table VII.

Results.

The third column of Table VII contains an expression of the pH gradient established by the electrolysis of the extract. As with the corn shoots, the pH of cell 1 (cathode)

Table VII.- Influence of effective concentrations of various fractions of extract of roots of germinated corn on the rate of reproduction of *Lemna*.

Culture Number.	Description of supplement* Source	pH (Glass electrode).	Kx 1000 From Fig.5	Condition of plants. Standard of comparison--plants grown in inorganic media. Kx1000 = 72.
686	Unelectrolyzed solution.	----	83	Slightly smaller fronds;lighter green color;slightly shorter roots.
	Cathode			
691	Cell 1	10.0	86	Very small plants;lighter green color;short roots.
694	Cell 2	5.1	86	Identical with 686.
695	Cell 3	4.1	92	" " "
696	Cell 4	3.6	92	" " "
699	Cell 5	3.3	88	" " "
700	Cell 6	3.1	83	" " "
701	Cell 7	2.9	96	" " "
702	Cell 8	2.6	95	" " "
	Anode			

* 4 cc. aliquot from each source was added to basal inorganic media. 4 cc. unelectrolyzed solution equivalent to extract of one root.

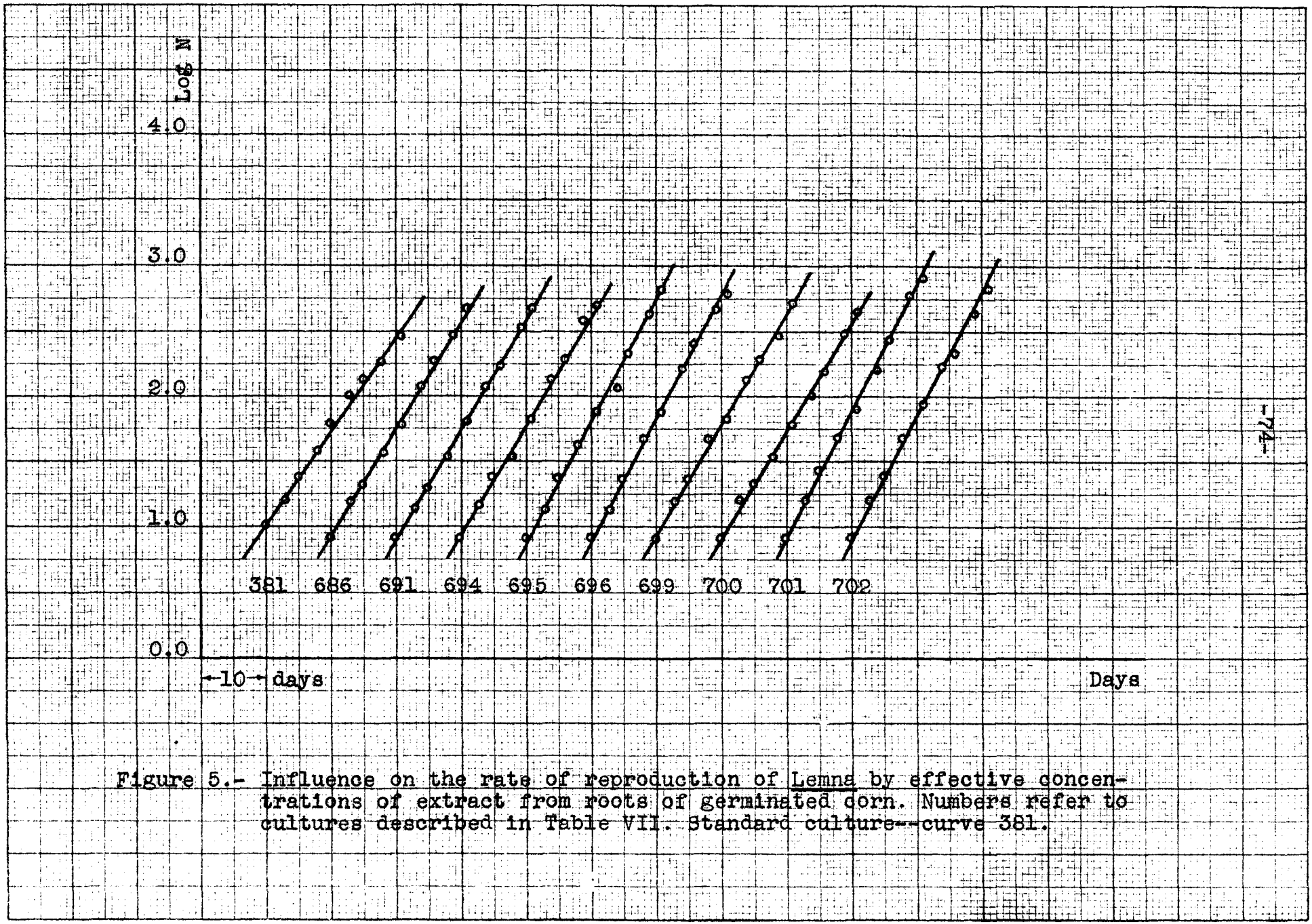

Figure 5.- Influence on the rate of reproduction of Lemna by effective concentrations of extract from roots of germinated corn. Numbers refer to cultures described in Table VII. Standard culture--curve 381.

was quite alkaline while that of cell 2 was distinctly acid; the difference between the pH values of the cells is 4.9. The pH of the remaining cells, 3 to 8 inclusive, was confined to a range of 4.1 to 2.6 with fairly uniform differences between cells.

There was very little change in the condition of the cells during the electrolysis. Small amounts of precipitated material which settled out, were found in the electrode cells.

The values for K for Lemna in the supplemented cultures are given in the fourth column of Table VII. These values, ranging from 83 to 96, show a distinct increased rate of reproduction for the supplemented cultures when compared with the 72 for the plants of the standard.

The rate of reproduction of Lemna in cultures supplemented from electrolyzed solutions shows that greater stimulation was obtained from two pairs of cells, 3-4 and 7-8, than was obtained from cells 1-2 and 5-6, and that all the electrolyzed fractions were either equal to or exceeded the unelectrolyzed solution in the stimulation of the rate of growth of Lemna. These results are difficult to explain. The greater stimulation obtained for cells 3-4 and 7-8 indicates a concentration of growth-promoting material by the electrolysis in these cells, and the concentration in the remaining cells of the series should therefore decrease, but on the basis of values for K there is no diminution of

the auxin in these cells for the rate of reproduction is very close to the 83 for K of the unelectrolyzed solution. The general stimulation of reproduction by the auxin is marked in all cases.

The condition of the plants in all the supplemented cultures compared with the standard showed similar changes at the end of the growth period. The fronds were slightly reduced in size and possessed a lighter green color; the waxy luster of fronds of plants grown in inorganic media was entirely absent.

The roots of plants in the supplemented cultures appeared healthy but showed a decrease in length when compared with the roots of Lemna grown in inorganic media.

General Discussion.

The similar pH gradient, which was present at the conclusion of the electrolysis of both the extracts of roots and shoots, was probably due to the phosphoric acid added to each solution before electrolysis. This is indicated in both cases by an alkaline reaction in cell 1 only and the marked increase in acidity of cell 2, compared with cell 1 (Tables VI and VII). It is doubtful if the small amount of extracted material contained in the pre-electrolyzed solution influenced the pH gradient, even if

the same growth-promoting materials were extracted from both shoots and roots.

Auxins have been shown to be present in corn seedlings by Dollfus (22) and Kornmann (43). The growth substance discovered by Leonian (45) in roots of germinated corn was found to be soluble in 95 percent alcohol. Auxins were found in the roots of oat seedlings, but in lower concentrations than in the coleoptiles, by Thimann (67). These findings, together with the widespread occurrence of auxins in plant materials, suggest that the growth materials obtained from corn roots which stimulated the reproduction of Lemna were also auxins.

Indication of a greater concentration of auxins in the shoots of germinated corn than in the roots is obtained from a comparison of the rates of reproduction of the Lemna in cultures supplemented with unelectrolyzed solutions; the extract from the shoots gave the greater rate of reproduction. This comparison checks the findings on auxins by Thimann for roots and shoots of oat seedlings.

That some other factor is operating in the case of the root extract or that there is a difference in the growth substances from the roots and shoots of corn is indicated by the action of the extracts in electrolysis. The treatment of the extract of roots appeared to concentrate the growth substance for Lemna in definite cells of the series; this is not shown by the electrolysis of the extract of shoots.

The decreased growth of roots of Lemna in solutions supplemented with auxins checks the results reported by other investigators. Boysen-Jensen (7), found the auxins completely stopped the growth of roots of beans, and Kögl, Haagen-Smit and Erxleben (41) reported a decrease in length of oat roots when grown in a solution containing auxin a.

Summary and Conclusions on Auxins.

A 95 percent ethyl alcohol extract of shoots and roots of germinated corn was used as a source of auxins. Water solutions of the extracts were treated by fractional electrolysis.

Under the growth conditions of the experiments, the rate of reproduction of Lemna was increased by the prepared extracts.

The auxins from shoots of corn were not concentrated in any of the cells by the fractional electrolysis; from the corn roots there was some indication of concentration, but no regularity. This lack of agreement in concentration obtained by the fractional electrolysis of the two extracts suggests that the growth substances from the two sources were not identical, although the effect on the Lemna was the same.

In both cases the extracted substances inhibited the growth in length of the roots of Lemna; this effect of auxins

has been reported by Kögl for roots of oat seedlings and by Boysen-Jensen for roots of beans.

The stimulation of the plants by the auxins was accompanied by a decrease in chlorophyll and a diminution in size of the fronds.

PURE COMPOUNDS .

The early investigations of plant hormones (auxins) made use of the tips of the coleoptiles of seedlings, principally those of Avena. These growth substances were secured from plants and the effects of the substances on different parts of the plants were observed. Since 1935 the attention of many investigators has turned toward experiments with similar synthetic compounds and their effect on the complete plant.

Davies, Atkins and Hudson (20), germinated the seeds of oats, mustard and cress under sterile conditions and found beta-indolyl acetic (synthetic heteroauxin) and beta-indolyl propionic acids retarded both the germination of seeds and the later growth of the young plants in concentrations as low as 10 mg. per liter. The compounds inhibited the normal growth of both roots and coleoptiles. Lane (44) also reported on the germination of oat seedlings in solutions of these acids. Root growth was measurably inhibited by concentrations from 0.032 to 50.0 mg. per liter of the compounds. Compared with the controls, the higher concentration decreased root growth in length ten times, and the inhibition was accompanied by a slight thickening and an increase in number of the roots. The treatment had no appreciable effect on the coleoptiles.Thimann (70), placed the roots of oat seed-

lings in solutions of beta-indolyl acetic acid ranging in concentration from 0.001 to 0.1 mg. per liter. After 48 hours the roots of controls were four times as long as those of seedlings in the lowest concentration of the acid and eight times as long as the roots of the seedlings in the greatest concentration. Thimann also reported a thickening of the roots with the decrease in length.

Leonian and Lilly (46) found that beta-indolyl acetic acid in concentrations of 0.1 to 1.0 mg. per liter inhibited the growth of detached roots and shoots of aseptically germinated corn grains, and higher concentrations up to 100 mg. per liter were toxic; the shoots were the less sensitive. These investigators concluded that beta-indolyl acetic acid was a growth inhibiting, rather than a growth inducing substance. They suggested that its action caused an accumulation of the growth promoting materials of the plant in the treated portion which eventually led to a weakening and ultimate death.

Marmer (51) grew wheat seedlings in nutrient solutions containing beta-indolyl acetic, beta-indolyl propionic and beta-indolyl butyric acids, using a wide range of concentrations. She secured no significant stimulation of the plants with the compounds; at an initial pH of 4.6 or 7.6 the substances decreased primary root, coleoptile and first leaf growth but increased the number of secondary roots.

The acids were more effective at the lower pH; at that acidity the concentration of beta-indolyl acetic acid which caused a decided reduction in growth of primary root was 0.012 mg. per liter, and was in the same range of concentration given by Lane and Thimann for inhibition of the roots of oat seedlings in a solution of the acid. Marmer secured a marked retardation of the growth of the wheat coleoptiles at pH 4.6, but Lane, using the acid in approximately the same concentration, reported no appreciable effect on the oat coleoptile. Thimann used lower concentrations and also reported no diminished growth of the coleoptile. Neither Lane nor Thimann reported on the growth of the first leaf of oats. Marmer (51) found the order of effectiveness of the acids changed at a pH of 7.6, beta-indolyl acetic acid being generally the most effective at the lower pH and least at pH 7.6.

Macht and Grumbein (50) secured a marked retardation of the growth of the roots of Lupinus albers seedlings when placed for 24 hours in solutions containing from 20.0 to 0.1 mg. of beta-indolyl acetic acid per liter; but when the roots of the seedlings were exposed for 15 minutes to solutions of lower concentrations of the acid, 0.001 to 0.0001 mg. per liter, a definite stimulation was secured in the succeeding 24 hours. These investigators compared the action of synthetic growth substances on plants to the action of drugs on higher organisms and considered the time of exposure a

very important factor.

In addition to the numerous articles on the action of these synthetic growth substances on plants in nutrient solutions, some investigators have reported on their effect on plants growing in soil.

Loehwing and Bauguess (47) applied 15 cc. of a water solution of 67 mg. of beta-indolyl acetic acid per liter to 118 day old Matthiola incana seedlings growing under greenhouse conditions in soil. Elongation of the stems of treated plants was noted at the end of the first day; the stimulation reached a maximum by the sixth day, but on the eleventh day the treated and control plants were equal. Greenfield (29) also experimented with seedlings of Matthiola incana in soil. He added beta-indolyl acetic acid in amounts ranging from 0.75 to 96 mg. per 1050 g. of soil. From 0.75 to 3.0 mg. gave no acceleration, 6.0 to 12.0 mg. accelerated growth until the sixth day, but in seven to fourteen days the treated and control plants were equal; higher concentrations were inhibitory. A loss of chlorophyll was noted for 24 mg. or more of the acid.

Grace (28) treated nasturtiums growing in sand, daily for 23 days, with 50 cc. of nutrient solutions containing from 0.01 to 2.5 mg. per liter of 1-naphthlene acetic acid. Plants to which solutions containing 0.01 to 0.1 mg. per liter were added were superior to the control. In another

experiment on eight weeks old lettuce in soil, this investigator reported an increase of 300 per cent in green weight of the tops by the addition of an equivalent of 150 mg. per acre of the acid.

Numerous investigations of the effect of synthetic growth substances on complete green plants have been carried out at the Boyce Thompson Institute. A review of this work is given in the section entitled "History". For the most part the investigations have involved the use of concentrations of organic compounds which induced stem bending, curvature of leaves, and initiation of adventitious roots on the stems and leaves of plants growing in soil or in water solutions. Responses were often secured in the plants of such an extreme nature that a continuation of normal growth was impossible; but, as pointed out, the experiments at the Institute have shown that synthetic growth substances are neither few in number nor confined only to one class of chemical substances.

The reported investigations show that synthetic growth substances produce many varied effects in complete plants. In general, these effects were observed during only a part of the life cycle of the plants, and microorganisms and organic matter, which may be factors in plant growth, were not always excluded. The healthy and normal growth of Lemna major, under sterile growth conditions and in the absence of

organic matter, provided an opportunity to investigate the effects of pure synthetic growth substances on one plant, in its complete form, and throughout its whole life cycle.

Compounds used and growth methods for Lemna.

Three organic compounds which were known to be growth promoting for green plants were chosen to determine the effects of synthetic growth substances on the rate of reproduction of Lemna. These were, beta-indolyl acetic acid, (synthetic heteroauxin), phenylacetic acid and phenylpropionic acid. The source of these compounds was noted at the end of the section entitled "Outline of Problem".

In preliminary experiments with these compounds in sterile inorganic media, irregular results were observed with Lemna. The irregularity seemed to be caused by the decomposition of the organic compounds in the weakly acid solutions during sterilization in the autoclave. Accordingly, water solutions of the compounds were sterilized separately by filtration under reduced pressure through Chamberlain Pasteur filter candles, and were added aseptically by means of sterile pipettes to the autoclaved standard solutions. The total volume was again 100 cc.

The initial pH of 4.7-4.8 was maintained in all cultures used in the experiments. Additional potassium hydroxide was added to the inorganic media to neutralize the

slight increase in acidity due to the organic acids. All determinations of pH were made with the glass electrode.

The procedure for the inoculation of the prepared media with Lemna was identical with that outlined in "Methods for Lemna" for pantothenic acid. The growth conditions were also similar except that the plants were transferred to fresh media twice a week and the light intensity in the growth chamber was increased from approximately 150 to 450 foot candles.

Experimental.

The compounds were added to the nutrient media in concentrations ranging from 0.0001 to 100 mg. per liter. Selected data, from duplicate cultures containing the lower concentrations, are recorded in Table VIII ; the curves plotted in Figures 6 and 7 represent the rate of reproduction of Lemna grown in the cultures described in the table. The first curve in each figure, curve 858 in Figure 6 and 861 in Figure 7, is for Lemna grown in standard inorganic media; data for these cultures are not given in Table VIII.

Results.

The values for the rate of reproduction, K, of the supplemented cultures are given in the fourth column of Table VIII. Compared with K = 85, for the Lemna grown

Table VIII.- Influence of various concentrations of pure compounds on the rate of reproduction of *Lemna*.

Culture Number.	Supplement: Compound	Supplement: Concentration. mg/liter of media.	Kx 1000 From Figures 6 and 7	Condition of plants. Standard of comparison--*Lemna* grown in inorganic media. For standard--Kx1000 = 85.
911	beta-Indolyl acetic acid.	0.01	85	Normal size; lighter green color, medium length roots.
912	" "	0.01	87	Identical with plants in 911.
913	" "	0.001	85	Normal plants.
914	" "	0.001	83	" "
915	" "	0.0001	85	" "
916	" "	0.0001	86	" "
917	Phenylacetic acid.	0.01	88	" "
918	" "	0.01	88	" "
919	" "	0.001	89	" "
920	" "	0.001	90	" "
921	" "	0.0001	86	" "
922	" "	0.0001	89	" "

Table VIII.- (Continued).

Culture Number.	Supplement: Compound	Supplement: Concentration. mg/liter of media.	Kx 1000 From Figures 6 and 7	Condition of plants. Standard of comparison--Lemna grown in inorganic media. For standard- Kx1000 = 85.
923	Phenyl-propionic acid.	0.01	90	Normal fronds; roots slightly shorter.
924	" "	0.01	87	Identical with plants in 923.
925	" "	0.001	88	Normal plants.
926	" "	0.001	90	" "
927	" "	0.0001	85	" "
928	" "	0.0001	90	" "

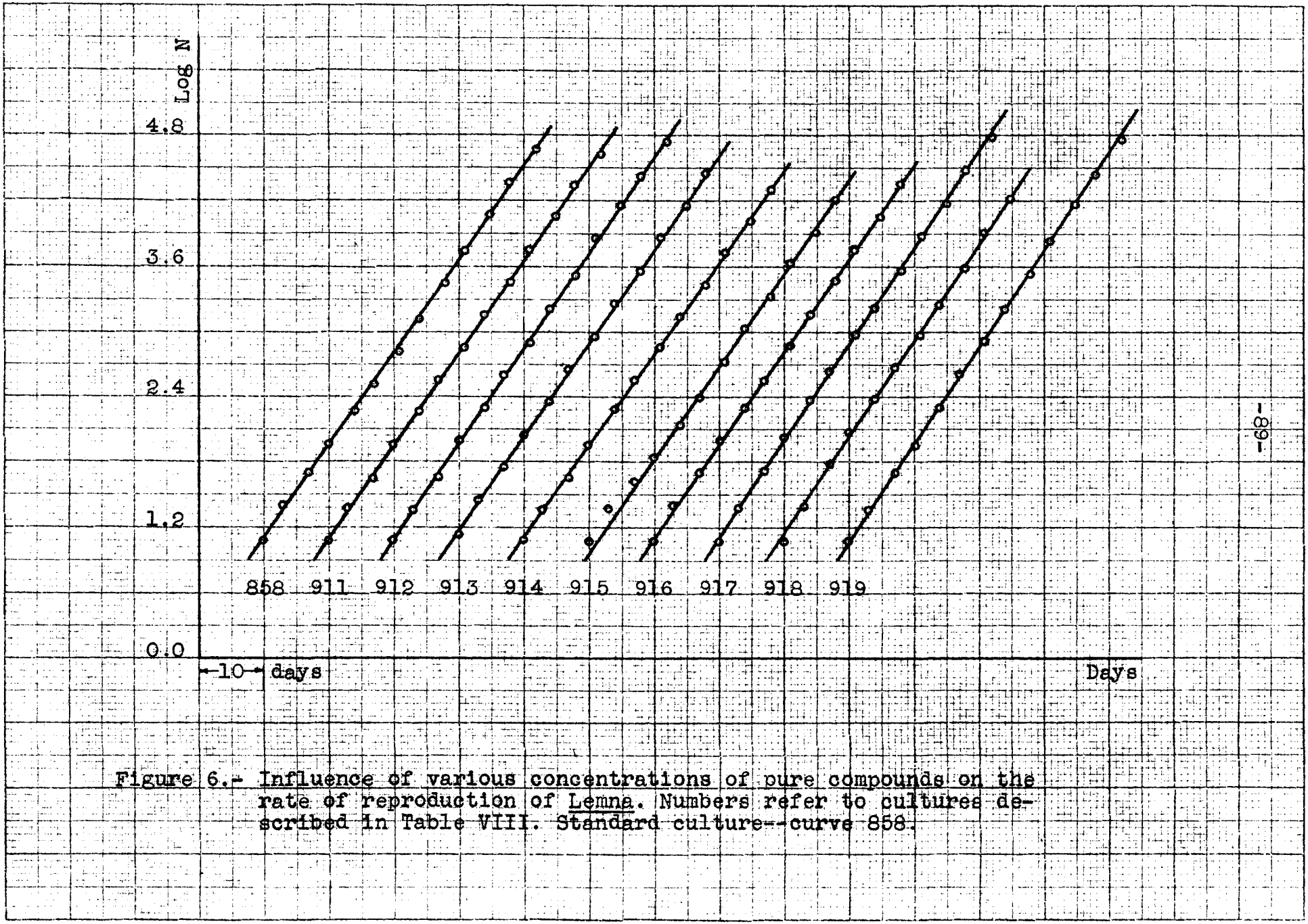

Figure 6.- Influence of various concentrations of pure compounds on the rate of reproduction of Lemna. Numbers refer to cultures described in Table VIII. Standard culture--curve 858.

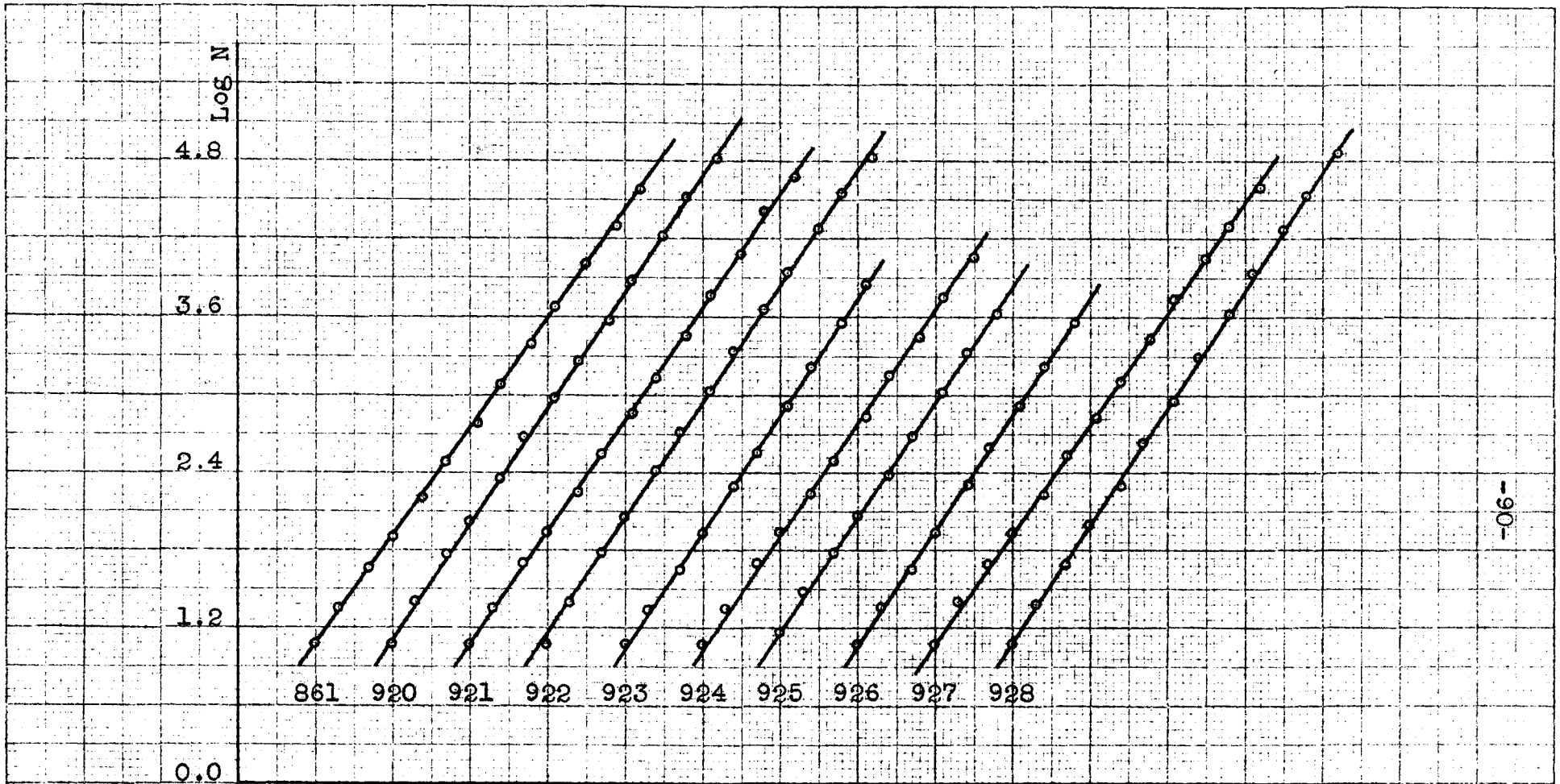

Figure 7.- Influence of various concentrations of pure compounds on the rate of reproduction of Lemna. Numbers refer to cultures described in Table VIII. Standard culture--curve 861.

in the standard, Lemna in cultures supplemented with 0.01, 0.001 and 0.0001 mg. per liter of beta-indolyl acetic acid were not stimulated in any case. Values for K show only a very slight stimulation for Lemna in solutions containing the same concentrations of phenylacetic and phenylpropionic acids; but no one concentration is better than any other.

The curves of Figures 6 and 7 represent the rate of reproduction of the plants and show graphically the same comparison recorded for the values of K. The curves for the cultures supplemented with beta-indolyl acetic acid (Figure 6) are all nearly parallel to the standard, while those for the cultures containing additions of phenylacetic and phenylpropionic acids (Figures 6 and 7) are in most instances only slightly steeper. The curves in Figure 7 for cultures 923, 924, 925 and 926 are shorter than the others, due to contaminations which caused the discontinuation of these cultures towards the end of the growth period.

A description of the Lemna at the conclusion of the experiment is given in the last column of Table VIII. Compared with the standard, the plants in all cultures were normal with the exception of the Lemna in duplicate cultures 911, 912 and 923, 924. The fronds of the plants in 911, 912 supplemented with 0.01 mg. of beta-indolyl acetic acid per liter, were normal in size but had a lighter green color; the roots of the plants were shorter in 927, 928, supplemented

with 0.01 phenylpropionic acid per liter.

Several temporary changes took place in the plants during the early part of the growth period. Fronds with slightly turned up tips were noted in the cultures containing concentrations of 0.01 mg. per liter of the compounds, but this effect was absent at the end of the growth period. Also, a distinct increase in size of fronds was obtained with Lemna in cultures 923 and 924, supplemented with the higher concentration of phenylpropionic acid, and this effect likewise disappeared by the end of the experiment.

The cultures described in Table VIII were supplemented with the lower range of the concentrations of the compounds; data from Lemna in the more concentrated solutions are not included with the selected material presented in the table and accompanying figures. Results with the higher concentrations of the compounds, up to 100 mg. per liter of media, are given below.

Concentrations of 0.1 and 1.0 mg. of beta-indolyl acetic acid per liter of media retarded the rate of reproduction of the Lemna. The effects of these concentrations on the plants were similar, differing only in degree. The fronds decreased markedly in size; they became a lighter green color and the edges showed a pronounced downward curvature. The connecting strands between the mother and daughter fronds were very long compared with those of Lemna in standard cultures. This

effect on Lemna was noted only in cultures supplemented with beta-indolyl acetic acid. The roots were very short in both concentrations. Ten mg. or more of the acid per liter of media killed the plants.

Concentrations of 0.1 and 1.0 mg. per liter of media of phenylacetic and phenylpropionic acid also retarded the rate of growth of the Lemna and inhibited root growth, but the effects produced on the fronds were different from those caused by identical concentrations of the synthetic hetero-auxin; this is shown by a comparison with Lemna in the standard medium.

The separate mature fronds of the plants in standard cultures were uniformly grouped in clumps of four; the inner ends of the fronds were slightly higher than the outer ends, giving an arched appearance to the group. The Lemna , in cultures supplemented with the concentrations of phenylacetic and phenylpropionic acids, had a more pronounced arched appearance of the clumps of fronds than the standard, and the separate fronds fitted snugly together. The fronds of the plants in the cultures supplemented with phenylacetic acid were slightly larger than the standard and possessed a darker green color; those of plants in cultures containing phenyl-propionic acid were normal in size and had a normal green color.

Concentrations of phenylacetic acid of 10 mg. or more

per liter of media were toxic. Plants in cultures containing that concentration of phenylpropionic acid became very small and had a light green color; the roots decreased to short, close clumped stubs. Quantities greater than 10 mg. per liter of this acid were toxic to the plants.

Although rather drastic physiological changes were effected in the Lemna growing in solutions containing high concentrations of the compounds, the plants recovered when transferred to inorganic media. Some of the very small plants, which resulted from treatment with 10 mg. of phenylpropionic acid per liter of media, were transferred to standard cultures. In the course of four weeks the plants appeared to be entirely recovered; the size and color of fronds and the roots were similar to those of Lemna of standard cultures and the normal rate of reproduction was resumed. During the first week after transfer to the inorganic media, the connecting strands of the mother and daughter fronds became very long, an effect previously noted only for Lemna in near toxic concentrations of beta-indolyl acetic acid. Similarly, plants transferred after three weeks in cultures supplemented with 1.0 mg. of beta-indolyl acetic, phenylacetic or phenylpropionic acids per liter, also showed a complete return to normal and healthy plants within a few weeks. Lemna from the solutions supplemented with phenylacetic or phenylpropionic acids increased in size during the recovery period, but within a few weeks they returned to

normal. No marked changes were observed during the recovery of plants transferred from the solution containing 1.0 mg. of beta-indolyl acetic acid per liter.

Discussion.

The reactions of Lemna grown in solutions supplemented with beta-indolyl acetic acid, in general check the results for seedlings of oats and wheat reported by Davies and co-workers (20), Lane (44), Thimann (70) and Marmer (51). The results obtained by Lilly and Leonian (46) for detached roots and shoots of corn, while not exactly comparable to those from complete plants, were of the same nature. The marked inhibition of root growth by the synthetic heteroauxin reported in all instances by these investigators was also obtained in the Lemna; the toxic effect upon the coleoptiles of seedlings secured by Davies, and on first leaf and coleoptiles by Marmer, was similarly produced on fronds of Lemna.

The suggestion of Leonian and Lilly that the acid is a growth inhibiting rather than a growth inducing substance appears to be well founded; if it is a phytohormone produced in plants during their normal life processes, then its production must be carefully controlled, as a constant external supply destroys the normal balance within the plant and brings about a detrimental effect. This is evident from

the recovery of normal health by Lemna when transferred from cultures supplemented with moderate concentrations of beta-indolyl acetic acid to standard inorganic cultures.

Indications are secured from the work of Macht and Grumbein (50) that under certain conditions the acid may be growth inducing. These investigators, as pointed out, reported a definite stimulation of the roots of Lupinus albers seedlings after a short exposure of the roots to very dilute solutions of beta-indolyl acetic acid. This suggests the possibility that short periods of intermittent contact for the plants with solutions of the acid might be beneficial. Although this was not indicated by Lemna transferred from solutions supplemented with this acid to standard media, some indication of such a possibility was secured when Lemna in solutions containing phenylacetic and phenylpropionic acids were transferred to inorganic media; in these two cases a definite increase in size of the fronds was observed.

The experiments with Lemna suggest that any marked stimulation produced in these plants, at least in solutions supplemented with the higher concentrations of the compounds, would be secured at the expense of some detrimental effect. This is indicated by the decreased rate of reproduction and inhibition of root growth, both of which accompanied the increased frond size of the Lemna grown in solutions containing 0.1 and 1.0 mg. of phenylacetic acid per liter of media. The

slight increase in the rate of reproduction of the Lemna, in solutions supplemented with the lower range of concentrations of phenylacetic and phenylpropionic acids, was the only stimulation effect obtained from the synthetic substances without some apparent harm to another part or function of the plant.

Summary and Conclusions on Pure Compounds.

Lemna were grown in sterile inorganic solutions containing additions of beta-indolyl acetic acid (synthetic heteroauxin), phenylacetic and phenylpropionic acids. Water solutions of each compound were sterilized by filtration through Chamberlain Pasteur filter candles and the solutions added aseptically to autoclaved nutrient solutions.

Under the growth conditions of the experiments 0.0001 to .01 mg. per liter of media of beta-indolyl acetic acid did not increase the rate of reproduction of Lemna, and identical concentrations of phenylacetic and phenylpropionic acids gave only a very slight acceleration to the rate of growth; higher concentrations of each of the acids retarded the rate of reproduction. The plants were killed by inoculation into solutions containing 10 mg. or more of beta-indolyl acetic or phenylacetic acids per liter of media, and by concentrations somewhat higher than 10 mg. of phenylpropionic

acid.

Phenylpropionic and beta-indolyl acetic acids were more effective root growth inhibitors than phenylacetic acid. The inhibition of root growth by beta-indolyl acetic acid was also reported by other investigators for seedlings of plants.

Phenylacetic and phenylpropionic acids in concentrations of 0.1 and 1.0 mg. per liter of media produced a slight increase in size of the fronds of Lemna; 10 mg. of the latter substance caused a reduction in size, with a loss of chlorophyll. Amounts of beta-indolyl acetic acid of 0.01 mg. or more brought about a marked decrease in frond size and were also accompanied by a loss of chlorophyll.

Lemna did not appear to be permanently injured by concentrations up to 10 mg. of beta-indolyl acetic and phenylacetic acids, and up to and including 10 mg. of phenylpropionic acid per liter of media. The plants, when transferred to standard inorganic media, resumed healthy and normal growth within a few weeks.

Any stimulation effects produced in the Lemna by the compounds were made at the expense of some part or function of the plant, with the exception of the very slight increase in the rate of reproduction produced by the lower range of concentrations of phenylacetic and phenylpropionic acids. The results appear to support the statement of Leonian and Lilly, that such substances tend to be more often growth inhibiting than growth inducing.

VITAMIN B_1.

The fact that minute traces of certain organic materials must be present in the diet of animals, including human beings, has been known for over twenty years. These organic substances, termed vitamins, although present in very small amounts in natural foods, have been proved to be absolutely essential. At first, in the study of these substances, plants were considered only as sources, but in the past few years definite evidence has been secured that some of the vitamins are essential factors in plant development.

Vitamin C (ascorbic acid) was the first vitamin to be used as a growth substance for plants. The experiments on the growth of peas in sterile cultures, supplemented with ascorbic acid, reported by van Hausen (31) and Virtanen (71) are reviewed in the section entitled "History". A marked increase in the dry weight of treated plants was obtained and Virtanen concluded that the vitamin was a phytohormone, indispensable to plants. Van Hausen (32) treated pea seeds for 24 hours with a solution containing 4 mg. per cc. of vitamin C. Seedlings germinated from these seeds and compared with controls, increased 35 percent faster in dry weight during initial stages of growth.

Bonner (4) reported a definite increased growth rate of excised pea embryos, of the variety "Perfection", when

5 parts in 1 million of vitamin C were added to the medium, but Kögl and Haagen-Smit (39) reported no influence of the vitamin in concentrations up to 0.8 mg. per embryo, on the growth of excised pea embryos of the variety "Kaapsche Groene" and "Kortstroo Schokkers". Bonner (4) suggested this lack of agreement might be due to a difference in the ability of varieties of peas to synthesize vitamin C for themselves.

Havas (33) germinated seeds of wheat, oats, tomato and paprika under sterile conditions in water solutions of vitamin C; the experiments were continued for 13 to 14 days. Concentrations of 1 to 5 parts in 10,000 of the vitamin gave no stimulation to the germination of wheat, but a definite acceleration to the growth of the seedlings; the shoots of treated plants showed 25 to 30 percent increase in weight compared with controls, and an increase of 50 percent was shown by the roots. A concentration of 2.5 parts in 1000 caused a slight check in germination of the wheat and a marked inhibition of the growth of the seedlings; a concentration of 5 parts in 1000 was toxic. Seeds of oats were much less sensitive than wheat, and for plants such as tomato and paprika, which are naturally high in vitamin C content, a concentration of 5 parts in 10,000 produced marked inhibitory effects. Adult tomato plants treated with 1 part in 10,000 of the vitamin showed no acceleration in growth or flowering, but the total weight of fruits produced was 20 percent greater

than controls. Davies, Atkins and Hudson (20) germinated seeds of oats, mustard and cress under sterile conditions in solutions containing one part in 10,000 of vitamin C; compared with controls, a stimulation of both germination and growth was secured.

Kögl and Haagen-Smit (39) demonstrated that vitamin B_1 is a growth factor for plants. They grew excised pea embryos in a sterile nutrient gelatin medium and found concentrations of crystalline B_1, as low as one part in 100 million, increased the length, weight and branching of roots. They recorded an increase in the length of shoots which was considered to be an indirect result of the greater root growth. Bonner (4), in a similar experiment, also secured stimulation of the growth of pea embryos; the optimum concentration of crystalline vitamin B_1 was found to be one part in 10 million.

Bonner (3) secured no new growth of excised pea roots in a sterile nutrient medium containing only essential salts and sucrose, but new root growth was obtained when a water extract of yeast was added to the culture, and even better growth when two parts in 10 million of crystalline vitamin B_1 was substituted for the yeast extract. Robbins and Bartley (57) and White (76) reported stimulation of the growth of excised tomato roots in sterile nutrient solutions supplemented with water extracts of yeast. Crystalline vitamin B_1, in concentrations of 0.1 to 1.0 mg. per liter of media, when substituted for the yeast extract, gave an increase in the growth

of the roots compared with roots growing in a solution of nutrient salts and sugar, but did not give an increase equal to that caused by the yeast extract. These results differ from those of Bonner (3) who secured greater growth of excised pea roots with B_1 than with the yeast extract.

Wildier's "bios", the complex growth factor for yeast, in which vitamin B_1 seems to be a fraction, appears to act as a growth substance for green plants. Kögl and Haagen-Smit (39) demonstrated that biotin, a bios fraction, stimulated the growth of shoots of pea embryos in sterile cultures but had no effect on the roots. A combination of biotin and vitamin B_1 gave a greater increase in growth than either alone, stimulating both shoots and roots. Bonner (4), secured a similar effect upon the shoots of the pea embryo using pantothenic acid, and also with a combination of the acid and crystalline vitamin B_1. Williams and his co-workers (77), who discovered this acid and used it principally as a yeast stimulant, have suggested that the substance may be a fraction of one of the water soluble vitamins. Bonner (4) believes it is probable that pantothenic acid and biotin are identical.

As sources of vitamins, plants and their growth conditions have been investigated from several angles. Following the discovery by Drummond and Zilva (23) that the vitamin A contained in cod liver oil originated in the diatom, a

unicellular algae in sea water, Jameson, Drummond and Coward (36), grew the organisms in artificial sea water containing only pure inorganic salts and free from all other microorganisms. The diatom synthesized vitamin A. Coward (18) successfully produced fresh water algae under sterile conditions on inorganic medium and in sunlight; these also produced vitamin A.

The effect of organic matter on the production of vitamins in higher plants has been shown by several experiments. Hunt (35) determined the vitamin B content of wheat grown on plots which had received varying treatment for a number of years. He found no difference in the quantity of vitamin B in wheat produced under different fertilizer treatment. On the other hand, McCarrison (49) found that wheat raised on soils receiving organic fertilizer in India contained more vitamin A than wheat raised on soils which received mineral fertilizer, and millet from organic fertilized soils had a higher vitamin B content than millet produced on mineral fertilized soils.

Viswa Nath and Suryanarayana (73) demonstrated that the effect of fertilizers on plants is carried on by the seeds produced. Seeds from plants grown on organic fertilized soil produced better plants than seeds from plants grown on mineral fertilized soils and their nutritive value was increased. Since the grain from the organic fertilized plots was richer

in both plant growth stimulants and vitamins, Viswa Nath suggested that the plant stimulant and vitamin were either the same, or else so related that the stimulant would enable the plant to form the vitamins.

The synthesis of other vitamins by higher plants has been shown. Evans and Hoagland (24) grew Canadian field peas in culture solutions under non-sterile conditions and demonstrated the synthesis of vitamin E in the plants. Virtanen, van Hausen and Saastamoinen (72) found that pea plants, grown under sterile conditions and in inorganic medium, produced carotene and vitamin C in practically the same amounts as those grown under natural conditions. This was production of vitamins by higher plants in the absence of bacteria and organic nutrients.

Lemna major was shown by Clark (15) and Frahm (27) to synthesize vitamin A, and by Frahm (27) to synthesize vitamin C.

The role of vitamin B_1 as a plant growth substance raised the question of the possible synthesis of the vitamin by Lemna when grown in inorganic solutions under artificial light, in the absence of organic matter and microorganisms. Lemna were available which had been grown under these conditions through hundreds of generations. If the vitamin is a phytohormone essential to this plant, then healthy and normal Lemna, growing in an inorganic medium and in the absence of microorgan-

isms, should synthesize it.

In the rat feeding experiments reported here to test the production of B_1, Lemna grown under the conditions described (inorganic media, absence of microorganisms and organic matter, and with electric light) are referred to as "sterile Lemna"; plants grown in a soil-water mixture, in the presence of microorganisms and organic matter and in sunlight, are termed "soil and non-sterile Lemna".

Preparation of Material.

The sterile Lemna were grown under the conditions described in " Methods for Lemna " in the Section on Pantothenic Acid, with the exception that the plants were subcultured at weekly intervals. Each culture received a sufficient number of plants to fill the flasks in a week. The excess Lemna were washed with two portions of distilled water, the moisture removed with filter paper, and the plants placed on separate papers in a dark compartment to dry in air. When the sterility of the cultures had been checked, the dried plants were placed in glass stoppered bottles and stored in the dark until needed for feeding to the rats.

The soil-water mixture used for the production of 'soil and non-sterile' Lemna consisted of 5 gm. of air-dry soil with 100 cc. of ordinary laboratory distilled water. The plants in this medium were grown in 250 cc. erlenmeyer flasks, loose-

ly stoppered with cotton plugs. The cultures were placed in a water bath at 25°C ± 0.5°, and given sunlight from a south window.

Due to the rapid growth of algae in these non-sterile cultures it was necessary to make transfers to fresh media twice a week. At the time of transfer, the excess plants from each flask were combined, washed in three portions of distilled water, and the excess moisture removed by placing the plants between filter papers; the prepared plants were then placed in a dark compartment to dry in air. When completely dry the plants were stored in glass stoppered bottles.

The preparation of plant material required approximately six months and nearly two months elapsed, after collecting the last lot of Lemna, before the dried plants were fed to rats; the prepared material varied in age, therefore, from two to eight months.

Experimental.

First rat feeding experiment.

Preparation of rats.

Young healthy rats from four to five weeks of age were selected from the rat colony of the Animal Nutrition Laboratory and placed on the basal vitamin B_1 free diet described by Chase and Sherman (10). This ration consisted of the

following constituents in percent by weight: autoclaved yeast 15, casein (extracted) 18, Osborne and Mendel's salt mixture 4, cod liver oil 1, butter fat 9 and starch, 53. The diet was used as described, with the exception that Crisco was substituted for the butter fat in the original diet given by Chase and Sherman. This fat was a more uniform product than butter and was less expensive.

The yeast used in the diet was prepared according to the procedure of Chase and Sherman (10). Northwestern yeast was placed in a steam autoclave and heated for two hours at 15 pounds pressure. This treatment was found by Chase and Sherman to destroy the vitamin B_1 in the yeast.

The casein was extracted by the method given by Sherman and Spohn (64). One liter of 60 percent by weight of alcohol was stirred for one half hour with 200 gm. of casein and then allowed to stand for five and one half hours. The alcohol was removed by filtration and the yeast washed on the filter with 500 cc. of 60 percent alcohol. The addition of one liter of 60 percent alcohol to the yeast was repeated with stirring and the mixture allowed to stand for 18 hours; after again filtering and washing, the yeast residue was washed finally with 500 cc. of 90 percent alcohol to facilitate air drying.

The basal ration was placed in feeding cups in the cages and the rats allowed to eat as much as they desired. The supplement to the ration was mixed with autoclaved yeast and water in proportions that would form a fairly thick suspension.

This mixture was fed by mouth to each rat in the supplemented groups by means of a graduated hypodermic syringe. The yeast was included in the mixture in order to secure a smooth suspension of the supplement in water and to add a substance, well liked by the rats, which would induce them to eat the test materials. The supplements to the basal diet included dry Lemna, the International Standard Preparation of vitamin B_1, and non-autoclaved yeast.

The dry Lemna plants were finely ground in a mortar and the powdered material mixed with the vitamin-free yeast and distilled water in such proportions that six grams of the mixture contained 500 mg. of the yeast and 500 mg. of the dry plant material. This quantity was fed daily to each rat whose diet was supplemented with Lemna.

The supplement containing the Standard vitamin B_1 was prepared in three concentrations. The International preparation consists of the anti-neuritic vitamin (B_1) adsorbed on kaolin. The International Unit, defined by the Department of Biological Standards, National Institute for Medical Research, Hampstead, London, is the anti-neuritic activity of 10 mg. of the International Standard Preparation. This amount, when included with a ration deficient in vitamin B_1 only, will enable a young rat of 50 to 100 gm. in weight to maintain normal health and growth and show an average weekly gain in weight of three grams over an experimental period of not less

than four, nor more than eight weeks. The standard was fed at three levels to three separate groups of rats. Each animal in the groups was given daily 6 gm. of a mixture of the standard preparation, autoclaved yeast and distilled water. The portion fed to each rat contained 500 mg. of the yeast plus either 2.5, 5.0 or 10.0 mg. of the preparation ; this provided groups maintained at levels of 1/4, 1/2 and 1 International Unit for comparison with the rats whose diet was supplemented with the dry Lemna.

Non-autoclaved yeast was added to the basal ration of one group of rats toward the end of the depletion period in order to provide a positive control group during the supplementation. This supplement consisted of a mixture of the non-autoclaved and autoclaved yeasts and distilled water. An amount of this mixture containing 200 mg. of the non-autoclaved yeast and 500 mg. of the autoclaved was fed daily to each rat of the group.

During the supplementation period the negative control group received daily per rat a volume of a mixture of autoclaved yeast and distilled water which contained 500 mg. of the yeast. This group, as well as the others, was allowed free access to the basal ration at all times.

Sufficient amounts of each supplement to last for a week to ten days were prepared and stored in a refrigerator at 32°F. These were warmed to body temperature before being

placed in the syringe and given to the rats. The small animals, when depleted of their vitamin B_1 reserve, could not consume the total volume of supplement at one feeding; this necessitated three or four feedings of each rat throughout the day.

After a depletion period of approximately one month the rats were divided into groups, and the various kinds of supplement added to the basal diet of the separate groups. One group of 12 rats continued to receive only the basal ration and functioned as the negative control.

The rats selected for the supplemented groups had steadily lost weight during the last half of the depletion period; they showed a rapid loss in weight in the three or four days before additions were made to the diet. The number of rats and the daily supplement of each rat in the group was as follows: Group 2 (12 rats), group 3 (12 rats) and group 4 (13 rats) receiving respectively 2.5, 5.0 and 10.0 mg. of the Standard vitamin B_1 preparation. Group 5 (1 rat) received 500 mg. of sterile Lemna, group 6 (1 rat) 500 mg. of 'soil and non-sterile' Lemna, and group 7 (4 rats) 200 mg. of non-autoclaved yeast. The last group were the first to rapidly lose weight during the depletion period; their quick recovery on the supplement of non-autoclaved yeast, rich in vitamin B_1, confirmed this vitamin as the controlling factor in their return to normal weight and

growth.

Due to the small amounts of each kind of Lemna only one rat was placed on those supplemented diets; these single animals were confined in the same cage with the negative controls in order to provide warmth and company for the young rats. Animals of the group which showed signs of weakening and possible death over-night, were removed to a separate cage. The small quantities of prepared plant material also stopped the supplementation period after 13 days.

The curves plotted in Figure 8 include for all groups the actual or the average gain and loss in weight per rat during the intervals of depletion, supplementation and discontinuation of supplementation.

Trends Indicated.

Curve 1 in Figure 8 shows the average loss in weight of the negative control group. The highest average weight of these animals was 84.7 gm. and during the progress of the experiment this decreased to 61.3 gm. At the conclusion of the supplementation period three of the animals of the negative group exhibited a marked lack of muscular coordination, and were separated and placed on a ration supplemented daily with 50 mg. per rat of the Standard vitamin B_1 preparation. On this diet the rats recovered normal locomotive powers and rapidly gained in weight. Curve 1a, Figure 8 ,

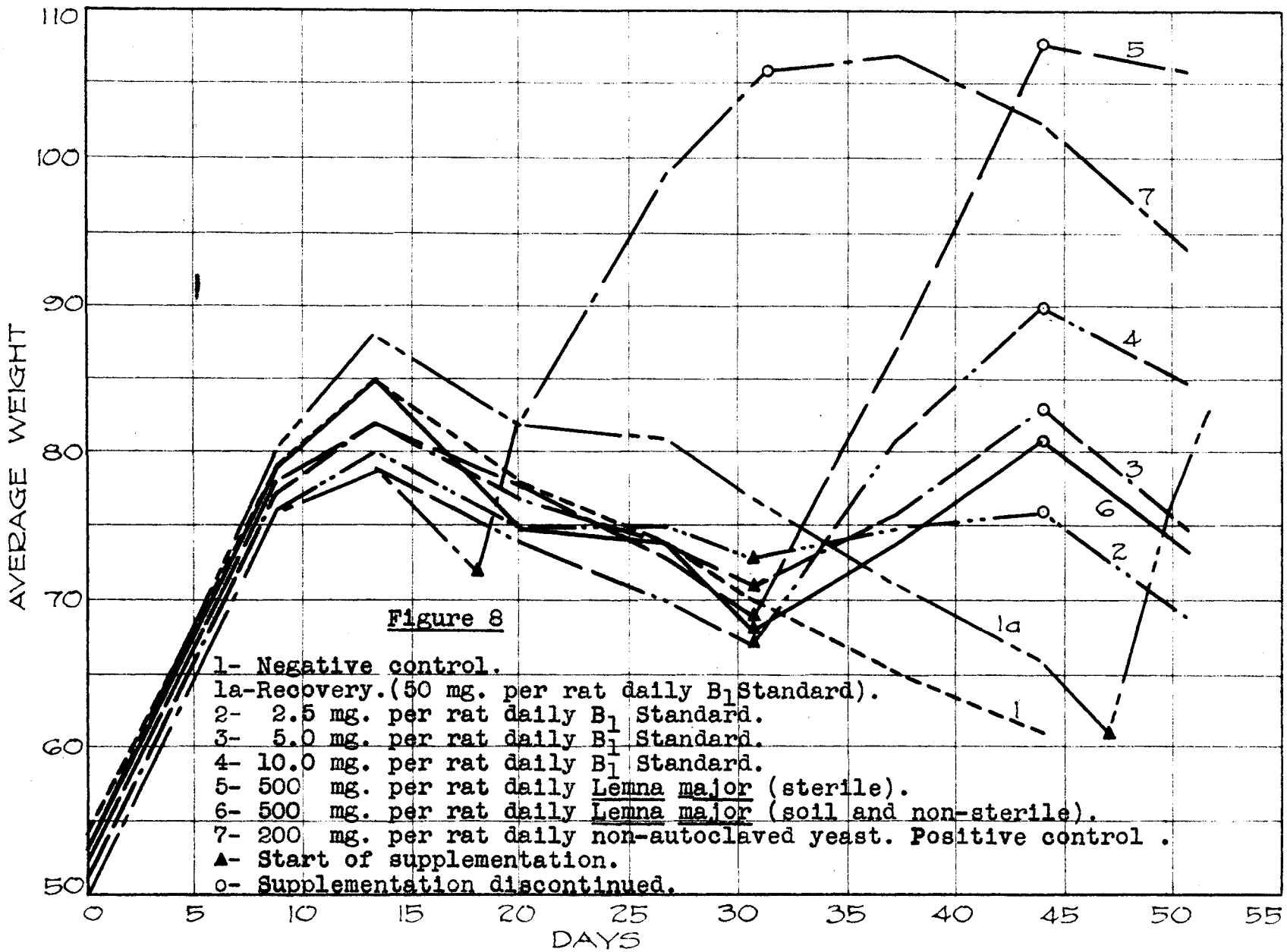

Figure 8

1- Negative control.
1a-Recovery.(50 mg. per rat daily B_1 Standard).
2- 2.5 mg. per rat daily B_1 Standard.
3- 5.0 mg. per rat daily B_1 Standard.
4- 10.0 mg. per rat daily B_1 Standard.
5- 500 mg. per rat daily Lemna major (sterile).
6- 500 mg. per rat daily Lemna major (soil and non-sterile).
7- 200 mg. per rat daily non-autoclaved yeast. Positive control .
▲- Start of supplementation.
o- Supplementation discontinued.

shows the average loss in weight of these rats on the basal ration and their rapid recovery on the supplemented diet.

The loss in weight of the negative control group with the development of muscular incoordination by three of the number, together with the return to normal of these impaired rats when a concentrate of vitamin B_1 was included in their diet, left no question regarding the lack of vitamin B_1 in the basal ration.

Curve 7 in Figure 8 represents the average weight of the rats of the positive control group; these rats were the first to develop symptoms of vitamin B_1 deficiency. As noted on the graph the basal diet of this group was supplemented early in the depletion period; at that time 200 mg. of non-autoclaved yeast was added to the diet. The group was returned to the basal vitamin B_1 free diet about the time supplementation was started for the other groups. The curve shows the rapid gain in weight of this group when the non-autoclaved yeast was added to the diet, and the loss in weight which followed the return of the rats to the basal diet; this checks the absence of B_1 in the diet shown by the negative control group.

Compared with the negative control group the two rats which received the Lemna supplement showed a marked increase in weight. This was especially true for the rat fed daily 500 mg. dry sterile Lemna; the curve for this rat, curve 5

in Figure 8, shows a rapid gain from a weight of 69 gm. to 108 gm. at the end of the supplementation period. The rat fed 500 mg. of dry Lemna (soil and non-sterile), curve 6, Figure 8, did not gain so rapidly; its weight increased from 68 gm. to 81 gm. during supplementation.

The gain in weight with a return to normal activity by both animals when fed Lemna supplement and the loss in weight when the supplement was discontinued, shows the presence of vitamin B_1 in the Lemna, with the larger quantity in the sterile plants.

Curves 2, 3 and 4 in Figure 8, show the average weekly gain of the rats on the three levels of vitamin B_1 standard. The rats in group 2, 1/4 unit level, gained an average of 3.2 gm. over the supplementation period. At this low level several of the animals became quite weak. The rats in group 3, 1/2 unit level, gained an average of 12 gm. per rat over the period, while those of group 4, 1 unit level, gained 24 gm.

A comparison of these values with those obtained for the rats whose diet was supplemented with Lemna indicates that in 500 mg. of dry sterile Lemna the quantity of B_1 is one and one half times that contained in 10 mg. of the International Standard preparation, while the amount contained in the 500 mg. of dry Lemna (soil and non-sterile) is equivalent to the B_1 in 5 mg. of Standard. These values are approximate only

and merely indicate that B_1 is present, as the gains resulting from the Lemna supplements are representative of only two rats, and also because of the short duration of the supplementation period.

From the results there was every reason to believe that the plants synthesized vitamin B_1 under both the different growth conditions; the experiment was therefore repeated with sufficient material to supplement the diet of a group of rats rather than a single animal.

Second Feeding Experiment with Rats.

Preparation of Supplement and Depletion of Rats.

The procedure used in growing and preparing the sterile and non-sterile Lemna was identical with that described under "Preparation of Material" for the first rat feeding experiment, with the exception that the non-sterile Lemna were grown in soil-water mixtures in large crystallizing dishes covered with watch glasses instead of in erlenmeyer flasks. The use of the dishes reduced the time required to transfer the Lemna to fresh cultures and to collect the excess plants for drying and storage. The prepared materials, therefore, were comparable to those used in the first experiment aside from a difference in age due to the unavoidable delay in feeding the dry plants to the rats. The dry Lemna used in the second experiment was stored for approximately

twice as long as the plant supplement for the first rat test.

In the second rat feeding test the basal vitamin B_1 free diet of Chase and Sherman (10) was replaced by a depletion ration of Evans, Lepkovsky and Murphy (25); this ration was made up in percent by weight as follows: extracted casein 30, sucrose 60, Osborne and Mendel's salt mixture 4. Sugar was the only source of energy. Both fat and starch were used as energy sources in the basal vitamin B_1 free diet of Chase and Sherman (10) but these substances have since been found to be undesirable dietary factors for vitamin B_1 assay tests. Guerrant, Dutcher and Tomey (30) reported that replacing starch by sugar in the vitamin B_1 free basal ration annulled the error from coprophagy in the assay of vitamin B_1. Starch was found to be incompletely digested by test animals; this could cause the formation of vitamins in the lower part of the digestive tract of the rat. Evans, Lepkovsky and Murphy (25, 26) eliminated fat from their basal diet because of the sparing action of this substance on vitamin B_1.

Casein used in the basal diet was extracted according to the method of Evans, Lepkovsky and Murphy (25). The substance was washed twice daily for five days with distilled water containing 1 cc. of glacial acetic acid per liter, and twice daily for two days with distilled water. It was then thoroughly drained by suspending in a cloth, and later dehydrated with alcohol and ether.

The rats were allowed free access to the basal diet at all times. Each day the first portion of the ration given all groups was supplemented with 500 mg. of autoclaved yeast and 50 mg. of cod liver oil per rat. The yeast was prepared as outlined for the first rat feeding experiment.

The vitamin B_1 supplement was incorporated with the basal ration during the period of supplementation. The syringe method of feeding the test material individually to the test animals (first experiment), proved to be a time consuming process and was not attempted for the second rat feeding test.

As the supplement was mixed with the basal diet the food consumption by the rats was checked daily; this made certain that all the supplement provided was eaten. The supplement, consisting of the vitamin B_1 test material and the mixture of non-autoclaved yeast and cod liver oil, was combined with a portion of the basal ration which was less than the amount consumed daily by the rats; when the daily amount of supplemented ration was entirely consumed, additional unsupplemented diet was placed in the cages for the remainder of that day.

Approximately one month was required to deplete the test rats of their vitamin B_1 reserves. Depleted animals which had shown a rapid loss in weight following a gradual loss were selected for the test rats. Litter mates were

placed in separate groups in all instances.

The grouping, together with the number of rats in the group and the average daily supplement per rat was as follows: 1a-(3 rats), negative control; 2-(5 rats), 5 mg. B_1 Standard or 1/2 unit; 3-(5 rats), 10 mg. B_1 Standard or 1 unit; 4-(5 rats), 20 mg. B_1 Standard or 2 units; 5-(4 rats), 250 mg. dry sterile Lemna; 6-(1 rat), 500 mg. dry 'soil and non-sterile' Lemna; and 7-(3 rats) positive control group, 1000 mg. non-autoclaved yeast.

The Standard Vitamin B_1 Preparation used was identical with that used in the first test; but the levels at which it was fed were changed from 1/4, 1/2 and 1 unit to 1/2, 1 and 2 units.

Results.

The average weekly loss and gain in weight of all groups of rats during the progress of the experiment is given by the curves in Figure 9.

The curves for group 1a, negative control, and group 7, positive control, show the depletion of the vitamin B_1 reserve of the test animals. This is evident from the rapid increase in weight of the rats when materials rich in B_1 were included with the basal ration. Daily supplementation of the diet of the positive control group with an average of 1000 mg. non-autoclaved yeast per rat was accompanied by an

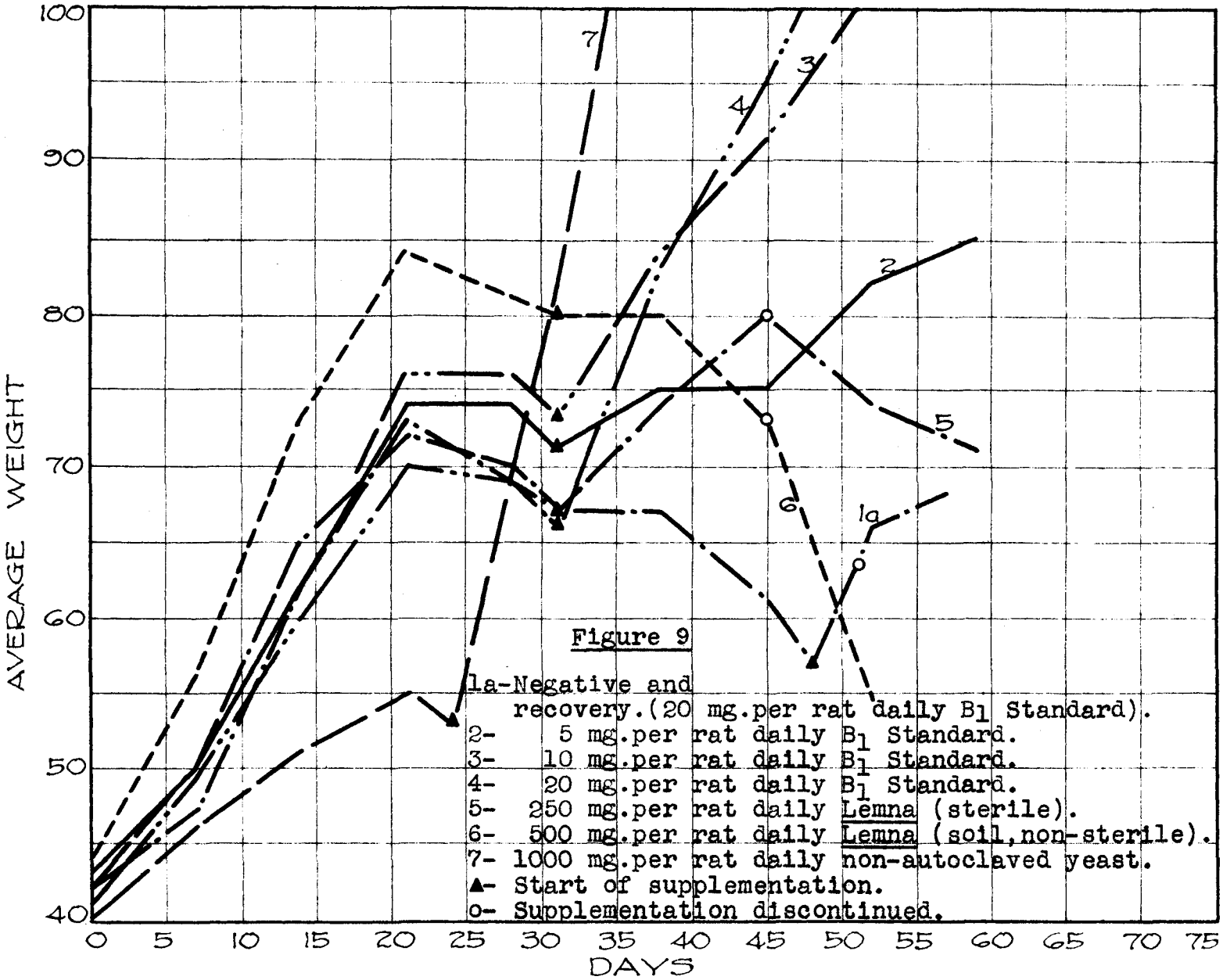

Figure 9

1a-Negative and recovery.(20 mg.per rat daily B_1 Standard).
2- 5 mg.per rat daily B_1 Standard.
3- 10 mg.per rat daily B_1 Standard.
4- 20 mg.per rat daily B_1 Standard.
5- 250 mg.per rat daily Lemna (sterile).
6- 500 mg.per rat daily Lemna (soil,non-sterile).
7- 1000 mg.per rat daily non-autoclaved yeast.
▲- Start of supplementation.
o- Supplementation discontinued.

average gain in weight from 53 gm. to 81.7 gm. in the first week. When 20 mg. of the vitamin B_1 Standard was fed daily for four days to each rat of the negative control group, following the period of supplementation of the test groups, these rats also gained rapidly in weight; the average weight of the rats increased from 57.3 to 66.3 gm. during the feeding of the standard material.

The average weights in grams of rats whose diet was supplemented with dry Lemna is shown by curves 5 and 6 in Figure 9. A comparison of these curves with curve 1a, negative control group, shows the presence of vitamin B_1 in the dry sterile Lemna, but very little if any of the vitamin in the dry 'soil and non-sterile' Lemna.

The rats fed daily an average of 250 mg. dry sterile plants increased steadily in weight during the supplementation period; when the supplement was discontinued the rats immediately started to lose weight (curve 5, Figure 9). Both these facts point to the presence of vitamin B_1 in the dry sterile Lemna supplement.

Curves 2, 3 and 4 in Figure 9, give the average gain in weight of the groups of rats whose diet was supplemented with the Standard vitamin B_1 Preparation. Curve 2 represents the group fed 5 mg. of the Standard or 1/2 unit; for the period of supplementation of the test groups (Lemna supplement) these animals gained an average of 3 gm. in weight or an

average weekly gain of 1.5 gm. The rats in group 3, (curve 3) which received 10 mg. or the 1 unit level, showed an average gain of 18.6 gm. for the same period or an average weekly gain of 9.3 gm., while the rats in group 4, 20 mg. of Standard or 2 unit level (curve 4) gained an average of 29.8 grams or an average of 14.9 gm. per week.

During the supplementation period the average gain per rat of the group fed dry sterile Lemna was 13.3 gm. or an average weekly gain of 6.7 gm. This gain is superior to the average weekly increase of 1.5 gm. in weight of the 1/2 standard unit group, but not quite equal to the average weekly gain of 9.7 gm. shown by the 1 standard unit group. The comparison indicates that 250 mg. of dry sterile Lemna contained an amount of vitamin B_1 greater than that in the 1/2 unit of standard and less than the vitamin in one unit of standard.

Discussion.

The second rat feeding experiment was carried out in an attempt to secure a quantitative measure of the vitamin B_1 produced in Lemna, but it proved to be impossible with the equipment at hand to produce sufficient plant material to carry out a rat feeding experiment, using groups of rats, for the minimum of four weeks required for standard vitamin B_1 assay, although by reducing the amount of daily supplement of dry sterile Lemna it was possible to feed that material to a

group of rats rather than a single animal. In the case of dry 'soil and non-sterile' Lemna each experiment was identical; 500 mg. of the material was used to supplement the daily ration of a single rat for a two weeks feeding test.

The response of the rats in the second experiment indicates a smaller amount of vitamin B_1 in both forms of Lemna supplement. Apparently some decomposition of the substance took place during the long storage period before the second assay, or possibly, the higher gains of the animals recorded for the first test were due to the individual characteristics of the single animals. Very little, if any vitamin B_1 was indicated for the second portion of 'soil and non-sterile' Lemna, although in the first experiment a small quantity was present.

Results of both experiments indicated that a smaller quantity of vitamin B_1 was synthesized by Lemna grown under non-sterile conditions in the presence of organic matter and in sunlight than was produced in sterile Lemna grown under electric light in an inorganic media. The intensity of light was constant for the sterile plants, while the growth of the non-sterile plants over the winter months was hindered by uncertain light because of short days and cloudy weather. Under these conditions, the non-sterile plants produced a large number of winter buds, some of which were collected with the plants and decreased the amount of green plant

material in the weighed supplement fed to rats.

The production of vitamin B_1 by Lemna under varied growth conditions was the reverse of results on vitamin B secured by McCarrison(49) and Viswa Nath (73) with millet on organic and mineral fertilized soils. But the only variable in their soil plot experiments was the soil treatment - applications of fresh manure against mineral fertilizer - while in the case of the Lemna , microorganisms and organic matter were excluded from the sterile cultures and the source of light was a variable factor. Under the conditions of the experiments the sterile Lemna certainly contained more vitamin B_1 than the non-sterile, but with the small amounts involved, the conclusion could not be drawn that this would always be the case.

Summary and Conclusions on Vitamin B_1.

Lemna major was grown, under varied conditions, for the production of plant material in sufficient quantities to supplement the basal ration of vitamin B_1-depleted rats in two feeding experiments.

Under the conditions of the experiments the response of the test rats showed that vitamin B_1 was synthesized by both sterile and by 'soil and non-sterile' Lemna; a greater quantity was produced by the Lemna grown in inorganic media under artificial light and in the absence of microorganisms

and organic matter, than in the plants grown in a soil-water mixture, in the presence of microorganisms and organic matter, and in sunlight; but the growth of the non-sterile plants was hindered by short and cloudy winter days.

An attempt was made to secure a quantitative measure of vitamin B_1 synthesized by the plants, by comparing the average weekly gain in weight of rats fed Lemna supplement with that of rats fed supplements of the Standard Vitamin B_1 Preparation at various levels. The quantity of dry Lemna available for either experiment proved inadequate for a standard assay period using groups of rats.

However, the comparison of the average gain in weight of rats on Lemna and Standard B_1 supplement, indicated for both experiments that 250 mg. of dry sterile Lemna contained slightly less of the vitamin than one unit of the Standard Preparation and for the first experiment that 500 mg. of the 'soil and non-sterile' Lemna were equivalent to one half unit of Standard, while the second experiment indicated the presence of still less of the vitamin. This decrease may have been due to decomposition of the vitamin during the long storage of the material before the second test; a slight decrease in potency was also noted for the second lot of sterile Lemna compared with the results from similar material in the first experiment.

Vitamin B_1 has been shown by other investigators to

be an accessory growth factor for some plants; its presence in both sterile and 'soil and non-sterile' Lemna suggests that the vitamins might also act as growth-promoters for Lemna.

General Discussion.

Both vitamin C (ascorbic acid) and vitamin B_1, have been found to stimulate plant growth and can be classed as accessory growth factors, for green plants. Vitamin B_1 appears to affect specifically the growth of roots; any increased growth of aerial parts has been considered an indirect result of increased root development. Vitamin C seems to stimulate the entire plant, its effect being confined to no one part. Bonner (4) pointed out that it was obvious that vitamins were not produced by plants merely to supply essential constituents in the diet of animals, and that probably the synthesis was for some definite function in the life of the plant. Viswa Nath (73) also indicated a possible identity of plant stimulants and vitamins. Under some conditions it seems evident the plant can make use of more of the vitamin than it actually produces.

The results from supplementation of the basal diet of vitamin B_1-depleted rats with dry Lemna, produced under varied growth conditions, leaves no doubt as to the presence of the vitamin in the plants. It was pointed out that the

plants also synthesize vitamin C (Frahm, 27). The synthesis of the greater quantity of both vitamins has been by sterile Lemna growing in inorganic medium, under artificial light and in the absence of organic matter; also, the plants under these growth conditions have the faster rate of reproduction. A similar correlation for vitamin C has been shown for peas grown under sterile conditions by Virtanen (71). This suggests a connection between the amounts of the vitamins synthesized by the plant and its rate of reproduction; it also indicates the possibility that the vitamins are accessory growth factors for Lemna.

SUMMARY .

Lemna major was grown under electric light, in the absence of microorganisms and organic matter, in inorganic solutions. The media were supplemented with concentrates of growth substances extracted from beef liver with 80 percent methyl alcohol (pantothenic acid); from roots and shoots of germinated corn with 95 percent ethyl alcohol (auxins); and with synthetic compounds known to be plant growth-promoters.

The concentrates of the growth substances were prepared by a fractional electrolysis of water solutions of the alcohol-soluble material.

In the electrolysis of the water solution of the liver extract the pantothenic acid moved toward the anode. The cathode cells also contained a growth substance, but the pantothenic acid caused a greater stimulation in the rate of reproduction of Lemna than the concentrates from the region of the cathode. The increased rate of reproduction of the plants from both fractions was accompanied by an inhibition of root growth and a decrease in both chlorophyll and size of the fronds. Ignition of the extracted dry matter destroyed the growth-promoting property.

The fractional electrolysis of the water solution of the extracts of shoots and roots of germinated corn did not

concentrate the extracted growth material from the shoots and gave irregular results with the root extract. A substance or substances, which increased the rate of reproduction of Lemna, were contained in both extracts. The stimulation was accompanied by an inhibition of root growth and a decrease in both chlorophyll and size of fronds; a similar effect was noted for the extract of beef liver.

Many pure compounds related to the auxins have been shown to possess plant growth-promoting properties. Three of these synthetic growth substances, beta-indolyl acetic, phenylacetic and phenylpropionic acids, in concentrations from 0.0001 to 100 mg. per liter of media, were tested on Lemna.

Beta-indolyl acetic acid is identical with hetero-auxin, one of the auxins produced in the coleoptile tips of Avena. This compound in any concentration used did not stimulate the rate of reproduction of the plants; 0.1 and 1.0 mg. per liter of media, caused a marked inhibition of root growth and a decrease in both chlorophyll and size of fronds, and 10 mg. or more killed the plants.

The lower concentrations (0.0001 mg. up) of phenyl-acetic and phenylpropionic acids gave a very slight increase in the rate of reproduction of Lemna, while 0.1 and 1.0 mg. per liter of media decreased that function and inhibited root growth but increased the frond size. Ten mg. per liter

of phenylacetic acid, and amounts somewhat higher than 10 mg. for phenylpropionic acid, were toxic to the plants.

The results obtained from the stimulation of Lemna by either growth-promoting extracts or pure synthetic growth-promoters indicated that any marked effects produced by these materials were at the expense of some other part or function of the plant. There is thus some justification for the view that such agents appear to be growth inhibiting rather than growth inducing.

The role of vitamins as accessory growth factors for green plants was discussed in relation to its possible connection with vitamin production by Lemna grown under varied growth conditions.

The synthesis of vitamin B_1 by Lemna was shown by the recovery of vitamin B_1-depleted rats fed a supplement of the air-dry plants. Lemna grown in inorganic media under artificial light, and in the absence of microorganisms and organic matter, produced a greater quantity of the vitamin than plants grown in a soil-water mixture, under sunlight, and in the presence of microorganisms and organic matter. The greater production of the vitamin B_1 in the Lemna was correlated with an increased rate of reproduction.

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