LECTURES *on* THE INORGANIC NUTRITION *of* PLANTS

By the same author:—

Relation of concentration and reaction of nutrient medium to growth and absorption of the plant (*Jour. Agr. Res. 18: 73-117, 1919*).

(with A. R. DAVIS) Composition of cell sap of plants in relation to absorption of ions (*Jour. Gen. Physiol. 5:629-646, 1923*).

The absorption of ions by plants (*Soil Science 16:225-246, 1923*).

(with A. R. DAVIS and P. L. HIBBARD) Influence of light, temperature, and other conditions on ability of Nitella cells to concentrate halogens in cell sap (*Jour. Gen. Physiol. 10: 121-146, 1926*).

Accumulation of mineral elements by plant cells (*Contrib. Marine Biol. Stanford Univ. Press 131-144, 1930*).

Absorption of mineral elements by plants in relation to soil problems (*Plant Physiol. 6:373-388, 1931*).

(With J. C. MARTIN) Absorption of potassium by plants in relation to replaceable, non-replaceable and soil solution potassium (*Soil Science 86:1-33, 1933*).

The plant as a metabolic unit in the soil-plant system. Essays in Geobotany in honor of Wm. A. Setchell (*Univ. of Calif. Press 219-245, 1936*).

(with T. C. BROYER) General nature of the process of salt accumulation by roots (*Plant Physiol. 11:471-507, 1936*).

(with W. H. CHANDLER and P. L. HIBBARD) Little-leaf or rosette of fruit trees. V. Effect of zinc on plants of various types in controlled soil and water-culture experiments. (*Proc. Amer. Soc. Hort. Sci. 33:131-141, 1937*).

Minute amounts of chemical elements in relation to plant growth (*Science 91:557-560, 1940*).

Salt accumulation by plant cells with special reference to metabolism and experiments on barley roots (*Cold Spring Harbor Symposia on Quantitative Biology 8:181-194, 1940*).

(with D. I. ARNON) Physiological aspects of availability of nutrients for plant growth (*Soil Science 51:431-444, 1941*).

LECTURES on the INORGANIC NUTRITION of PLANTS

(Prather Lectures at Harvard University)

BY

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PREFACE

These lectures on the Inorganic Nutrition of Plants given at Harvard University, under the Prather Lectureship, were not originally intended for publication. Suggestions were later made that these brief reviews might have an interest for a wider audience. Dr. VERDOORN was sufficiently impressed by this point of view to undertake publication of the lectures. They have been revised and two supplementary lectures added.

Obviously, this small volume cannot have any of the characteristics of a monograph or of a text. Its purpose is to present a general perspective of several important aspects of the field of plant nutrition, with a broad interpretation of this term in mind. It is hoped that some of the problems of the soil-plant system awaiting further exploration may be brought into focus.

Underlying the plan of these discussions is the thought that some students of the plant sciences, especially among those who are not primarily concerned with the literature of plant nutrition, may find it worthwhile to gain such impressions of this complex field of study as can be offered in highly compact form.

Most of the illustrative material is drawn from the experiences of several groups of Californian workers. The assumption was made that in lectures of the present type and objective the writer should emphasize the work with which he has had the most direct contacts. The limited scope of the lectures precluded consideration of a vast number of important contributions to the field surveyed.

I wish to express my deep appreciation to T. C. BROYER for his assistance in the preparation of the illustrative material and for other assistance. I am also grateful to D. I. ARNON, H. S. REED, J. C. MARTIN, and W. P. KELLEY for making available certain of the illustrations in original form.

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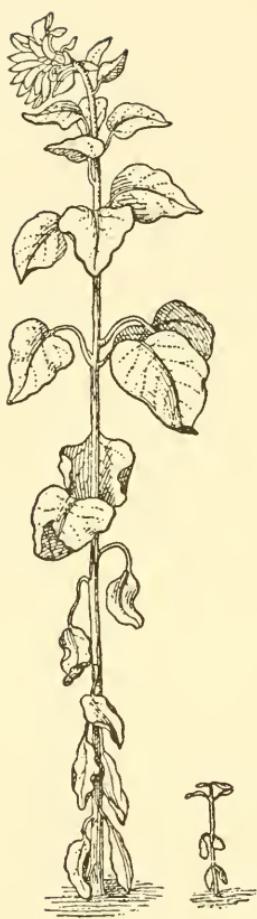
A SURVEY OF PROBLEMS OF PLANT NUTRITION

Introduction: — I deem it an honor as well as a great pleasure to receive the invitation to deliver these lectures at an institution that has made such distinguished contributions to plant science. I am sure that the only reason you have thought that my discussions could bring you any profit is that I happen to be a member of a group of research workers who have sought over many years to increase our knowledge of the nutrition of plants. There is implied in this statement recognition of an essential condition of satisfactory progress in the field of study of Plant Nutrition, if this term is used in the wide sense I wish to give it for my present purpose. This condition is the uniting together, although not according to any rigid pattern, of persons of varied technical interests, in a research program that has a common objective. The reasons why this concerting of effort and coordination of experience in the study of Plant Nutrition is so needful will, I hope, emerge from my series of lectures.

Subjects pertaining to Plant Nutrition have not, I understand, been previously presented here in a lectureship like the one I now occupy and there may be found, therefore, some reason for a very brief outlook at the nature of the system that confronts the student of Plant Nutrition. I doubt that there exists in any other system presented for scientific or prac-

tical study a comparable degree of complexity. Indeed, scientifically considered, this complexity occasionally takes on an almost appalling aspect.

The multiphase system which the investigator of plant nutrition explores is that of the soil-plant-atmosphere with its innumerable interrelations and inter-reactions. Inherent in the green plant itself are all the complexities common to living organisms and to these must be added the complexities of the soil medium in which the plant is anchored and finds some of the substances essential for its nourishment. The soil is a natural body, the product of geological and weathering agencies. It is extremely variable from place to place, also changing with depth, and is composed of inorganic and organic matter of utmost chemical and physical diversity, existing in many states of subdivision, including those states of extremely fine division that confer the properties of colloidal behavior. The soil is not a dead or static system. It is the nourishing medium of remarkably varied forms of plant and animal macro- and micro-organisms, whose life activities continuously modify the non-living part of the soil and reciprocally are modified by it. Finally, the whole system of plant and soil is subject to the influence of another constellation of factors in the atmospheric environment: light, temperature, humidity, rainfall, air movement, as well as carbon dioxide and other gaseous components of the atmosphere. Under natural conditions these are usually uncontrolled or uncontrollable factors.

To envisage further the general nature of the relation of the environment to the development of the green plant, it is first necessary to recall the special functions of organic synthesis characteristic of this kind of plant. From the simplest chemical substances, of which carbon dioxide from the low grade source in the atmosphere, and water from the soil, are quantitatively most important, organic compounds are cre-

ated, including those of the highest degree of intricacy and of largest molecular weights among organic molecules. The familiar fact will be recalled that upon this synthetic power and upon the processes by which the energy of sunlight is absorbed and fixed all life is ultimately dependent. The green plant winds up living systems as a whole, which otherwise would run down and disappear.

Even in a single microscopic green cell reside potentialities for chemical reactions of extraordinary diversity. Smoothly, rapidly, at low temperatures, without the strong reagents of the chemist, the cell carries on its remarkable syntheses. The chemical compounds built up from the simple substances are generally of the finest specificity. We think of giant protein molecules of biologically determined architecture, and we remember that even relatively small molecules, like those of the sugars, may exist in right and left handed forms of equal chemical stability, with as nearly as possible an even chance that either form will be synthesized unless a directional agency intervenes; and that the plant cell nearly always synthesized only one of the forms. Some investigators believe that this is invariably true of the molecule freshly synthesized by the protoplasm, although under some circumstances, a reversion may later occur to racemic forms. All this is, of course, not new knowledge. There are, however, several aspects of the functions of plant cells that have been illumined by the new kind of experimental evidence that can be gained through use of the tool of chemical isotopes, both stable and radioactive forms. It appears from recent work by several groups of investigators that the chemical organization of either the metabolizing plant or animal cell is far more dynamic in nature than was previously imagined. The concept evolved is that of a continuous flux of interdependent chemical processes, with complex molecules built up and broken down with great rapidity, all under the orienting

influence of biological catalysts, the enzymes, which are themselves large and exceedingly complex protein molecules.

The study of plants growing under a natural or agricultural environment of necessity must encounter all the factors of the system, soil-plant-atmosphere, of which I have spoken. Viewing the field as a whole, the attack on problems of plant nutrition assumes wide dissimilarity of method. At one time, the appropriate tool may be a spade or a soil auger; at another time a highly specialized and refined tool of physics or chemistry may be required. For some purposes crude measurements in terms of pounds or ounces may suffice; for other purposes, a thousandth of a milligram is of consequence, or even much less than that, in the determination of the effects of certain plant hormones in relation to nutritional or physiological processes of the plant.

Problems of the Soil Solution in Relation to Plant Nutrition: — I shall not carry further these generalizations but at once embark on my discussion of specific problems of plant nutrition. I wish to begin with the early researches, in a period now about twenty-five years past, of the laboratory with which I am connected. Some justification for this procedure may be offered, in that it will afford an opportunity to indicate the underlying reasons and the stimulus for undertaking much of the experimental work to be described in the subsequent lectures. At the same time some of the general trends of modern developments in the investigation of plant nutrition in certain of its aspects can be brought to your attention, and perhaps placed in a useful perspective.

In a period shortly preceding that of which I have spoken, several investigators had issued a series of publications in which were set forth several far-reaching conclusions concerning the nutrient interrelations of soil and plant which appeared opposed to prevail-

ing views of agriculturists and which aroused the sharpest controversy. In their more extreme form at least, these conclusions embodied the concept that the liquid phase of the soil, the soil solution, and according to then prevailing theory the immediate source of the plant's inorganic nutrients, was a dilute solution more or less similar in composition for most soils. The reasoning was in part that soils are all extremely heterogeneous, all containing relatively large amounts of the principal soil minerals, and that a solution saturated with respect to these minerals would be present in the majority of soils. The effects of fertilizers were ascribed often to factors other than that of modifying the soil solution as a nutrient medium for plants. Great stress was placed on the presumed presence in infertile soils of toxic organic substances in minute amounts. Fertilizers, it was thought, might frequently act by overcoming in some way the effects of these toxic substances.

All this is now, of course, only of historical interest. Certain fallacies in the view I have outlined have long been apparent. Nevertheless, these early discussions had valuable consequences. For too long had the thinking of students of plant nutrition been influenced by the doctrine of statics as enunciated by LIEBIG — that the fertility of a soil rose and fell in exact proportion to the mineral nutrients withdrawn from or added to the soil. The discussions to which I refer argued strongly for a dynamic interpretation of the soil system and this was sound despite erroneous teachings as to the particular nature of the system.

This, then, was the general background of our own initial researches in plant nutrition, inaugurated under the direction of Professor J. S. BURD, associated with G. R. STEWART and others. Much had been written about the soil solution and controversy had not ceased. It was apparent that experimental evidence was most inadequate. Generally, chemical analyses had been made on field samples of soil under no adequate con-

trol. Following a search of the literature, it was de-
cided that a useful service might be rendered to plant
nutrition through a systematic and well-controlled in-
vestigation of soil solution phenomena. For this pur-
pose 13 soils in large lots were assembled at the cen-
tral laboratory. These soils were selected from diverse
locations in California and with reference to particular
soil types. After thorough mixing, the soils were
placed in duplicate tanks holding about a ton each.
These soils are still in course of study after twenty-
seven years. Barley crops have been grown on the
soils during this period except for certain years in
which some soils were allowed to lie fallow. Only
distilled water has been added to the soils and drain-
age has been prevented. The water is added in such
amounts as to avoid an excess. No crop residues or
nutrient salts have been returned to the soils, save
for the finer parts of the root system which could not
be removed. (*See plate 1*).
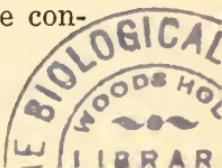
Chemical studies of many types have been made
on the soils and on the crops produced. I suppose
that no other soils held under similar control have
received such extensive examination over so long a
period of time. I am now concerned with only a few
aspects of these experiments, especially those relating
to the soil solution. The first objective was to follow
the seasonal and secular trend of changes in the com-
position of the soil solution and the effects of cropping
on its composition. For some time the only technique
available was that of making water extracts of the
soils, but later a procedure was developed (BURD
and MARTIN, 1923), following a suggestion of F. W.
PARKER (1921), for obtaining solutions from the soil
at moisture contents approximating the general range
of moisture contents held by the soil as the crops
grew.

The general procedure was to pack the soil care-
fully in a brass tube, then to place over the soil a
column of water and subject the system to air pres-

sure. The liquid that issued from the bottom of the tube was collected in fractions and on each fraction a conductivity measurement was made. Those fractions of nearly constant conductivity were assembled and mixed for chemical analysis. When a reduction in the conductivity value occurred, indicating a dilution of the soil solution, additional fractions of solution were discarded. Displaced soil solutions are of course composite in nature and reflect only an average composition. (*See plate 2*).

To bring the discussion up to date, it may be added that during the past few years, RICHARDS (1941) and his collaborators, at the Federal Salinity Laboratory, Riverside, California, have devised another technique for obtaining soil solutions. This is accomplished by a pressure membrane apparatus, in which no displacing liquid is used. By this technique, solutions may be obtained from soils at very low moisture contents. Also heavy soils, difficult to deal with by the displacement method described above, can be more conveniently subjected to displacement.

The early experiments yielded data of much interest at the time; concerning first, the ranges of concentration of various ions and their proportions in the soil solutions of these initially productive soils, and second, the striking effects of seasonal biological activities and of the absorption of nutrients by the crop plants (barley) on the composition of the soil solution. Except for sulphate and bicarbonate, cropping reduced the concentrations of the principal ions, initially present.

Comparisons of the soil solutions of different soils at a given time and of the same soil at different periods of sampling, allowed the conclusion to be drawn that a soil solution is not a simple saturated system of minerals and water, but instead is an ever-changing system, biologically controlled by the activities of soil microorganisms and of the higher plants growing in the soil. The different ions did not behave alike. Their

relations to the solid phase of the soil were different and also their relations to the selective absorption of ions by living root cells. Some ions fluctuated in concentration more widely than others.

The phosphate ion was present usually in very low concentrations. Potassium fell in an intermediate position. The total amounts of phosphate ions present

Solutions displaced from cropped (A) soils at beginning and at the end of the growing season (1923) and at the beginning of the next growing season (1924)

SOIL NUMBER	DATE	MOISTURE	pH	PARTS PER MILLION OF DISPLACED SOLUTION							
				NO_3	HCO_3	SO_4	PO_4	Ca	Mg	Na	K
		per cent									
7	Apr. 30, 1923	10.7	7.4	174	83	655	1.1	283	106	49	24
	Sept. 4, 1923	12.5	7.6	58	155	432	0.6	193	47	40	9
	Apr. 28, 1924	14.2	7.6	222	142	571	0.6	296	67	52	11
8	Apr. 30, 1923	9.6	7.4	274	93	633	2.5	267	93	31	20
	Sept. 4, 1923	8.4	7.6	88	143	275	1.4	153	56	28	11
	Apr. 28, 1924	13.2	7.2	227	107	441	2.1	232	78	23	10
9	Apr. 30, 1923	11.4	7.6	160	73	432	0.9	213	75	27	12
	Sept. 4, 1923	13.8	7.8	43	167	121	0.3	107	32	30	7
	Apr. 28, 1924	12.9	7.6	182	98	390	0.5	220	57	28	10
10	Apr. 30, 1923	13.9	7.2	230	40	360	1.2	187	145	23	33
	Sept. 4, 1923	14.4	7.6	40	112	241	0.7	108	38	16	23
	Apr. 28, 1924	16.0	7.2	200	73	423	1.1	216	70	17	25
11	Apr. 30, 1923	12.9	8.2	166	160	645	3.3	213	93	84	39
	Sept. 4, 1923	12.4	7.6	16	234	598	1.2	192	64	44	22
	Apr. 28, 1924	11.4	8.1	286	259	854	2.9	300	102	85	38
12	Apr. 30, 1923	12.1	7.3	115	40	281	2.3	120	46	27	13
	Sept. 4, 1923	13.5	7.6	50	68	184	0.7	80	28	12	9
	Apr. 28, 1924	13.7	7.3	156	49	313	0.7	156	50	18	10
14	Apr. 30, 1923	18.0	8.0	146	107	295	1.3	140	47	56	45
	Sept. 4, 1923	18.7	7.6	13	176	174	0.2	80	23	26	23
	Apr. 28, 1924	18.8	7.6	167	142	380	1.0	168	55	47	49

TABLE 1. — Composition of solutions displaced from cropped soils at beginning and end of growing season and at beginning of next growing season. (From J. S. BURD and J. C. MARTIN, 1924).

in solution in the whole mass of the soil, at the beginning of the season, were far short of accounting for the amounts absorbed by the crop during its growth period. On the assumption that the soil solution was the immediate source of all the nutrients absorbed by the plant,* it was necessary to conclude that as plants grew and absorbed phosphate, this ion had to be re-

*An additional mechanism will be presented at a later time.

placed many times in the soil solution, from the solid phase of the soil, and to varying degrees the same was true of other ions. The concept of "supplying

Displaced solutions from cropped (A), fallowed (B), and air-dry stored (S) soils after 8 years
(Results from table 1, calculated to a uniform moisture basis for each soil)

| SOIL	MOISTURE	pH	PARTS PER MILLION OF DISPLACED SOLUTIONS										
			NO$_3$	HCO$_3$*	Cl	SO$_4$	PO$_4$*	SiO$_3$	Ca	Mg	Na	K	Total
	per cent												
7A		7.4	116	83	0	438	1.1	—	189	71	33	16	947
7B	16	7.6	1781	73	55	454	1.2	—	672	134	75	38	3281
7S		7.2	1468	69	313	184	3.3	38	547	112	123	39	2896
8A		7.4	179	93	0	413	2.5	—	174	61	20	13	955
8B	14.7	7.0	1798	59	41	445	4.0	—	590	160	68	77	3242
8S		6.9	2182	77	260	451	7.2	61	539	204	156	156	4093
9A		7.6	121	73	0	326	0.9	—	161	57	20	9	750
9B	15.1	7.6	966	63	37	428	1.2	—	469	95	54	30	2144
9S		7.3	680	46	372	184	2.9	33	380	75	88	21	1882
10A		7.2	173	40	0	270	1.2	—	140	109	17	25	775
10B	18.5	7.0	1658	34	31	339	2.1	—	532	132	46	72	2846
10S		6.8	937	34	348	148	4.0	51	360	91	115	89	2173
11A		8.2	138	160	0	537	3.3	—	177	77	70	32	1200
11B†	15.5												
11S		7.8	1049	171	367	344	12.1	49	407	128	203	100	2839
12A		7.3	92	40	0	225	2.3	—	96	37	22	10	524
12B	15.1	7.0	1473	34	39	330	0.3	—	573	148	59	44	2701
12S		7.3	208	39	80	170	3.1	48	145	48	52	30	823
14A		8.0	142	107	0	287	1.3	—	136	46	54	44	817
14B	18.5	7.4	1709	54	58	384	1.7	—	529	135	82	114	3069
14S		7.4	779	78	100	188	4.3	54	290	91	74	89	1747

* Bicarbonate and phosphate remain as in table 1 (see text.)
† Soil not available for study.

TABLE 2. — Composition of displaced solutions from cropped, fallowed and original soils, after 8 years. (Results calculated to an approximately uniform moisture basis for each soil). These data illustrate the general magnitudes of concentration, for different ions present in the soil solutions of the soils discussed in the text. The so-called fallowed soils were actually cropped the first year and were cultivated and irrigated the first four years. The remaining four years they received no treatment other than irrigation (distilled water). The principal interest of the table for present purposes is to give an idea of the relative concentrations of different solutes and the effects cropping have had on these concentrations. The value for the stored and fallowed soils, S and B series should be compared with those for the A series. Note the differences in magnitudes of concentrations for NO$_3$, K and PO$_4$. (From J. S. BURD and J. C. MARTIN, 1924).

power" of the soil had to be invoked to understand the nutrient capacity of the soil. Soil solution data alone were inadequate to explain the potentiality of the soil for delivering nutrients to the plant over extended periods of time. As the years passed, soil

solution studies were made on many other soils in California and elsewhere, and it has become apparent that soil solutions can often be much more dilute than those of our original set of soils, and still plants will not necessarily fail to absorb adequate amounts of nutrient ions. Thus, the concept of "supplying power" and the interrelation of the solid to the liquid phase of the soil became considerations of paramount significance and they are so today.
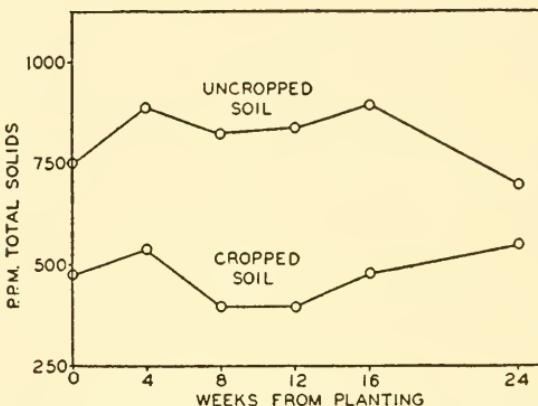

TEXTFIGURE 1. — Effect of cropping on the soil solution as reflected in total solids of water extracts of the soil at various periods of crop growth. (From HOAGLAND, 1918).

In these experiments prominence must be given to the nitrate ions which undergo the greatest changes in concentration in the soil solution.* With continuous cropping the capacity of the soils to produce barley crops greatly diminished, so that most of the soils are now at a low level of production. Primarily responsible for the decreases in yields seems to be the increasingly inadequate supply of nitrate nitrogen, although the possibility exists that other limiting factors may be entering, particularly, in certain soils,

*Nitrate ions may be selectively absorbed by plants and replaced in the solution by bicarbonate ions. Also see later discussions.

insufficiency of supplying power for phosphate. The design of the investigation has prevented thus far a specific test of this point.

During the first few years of cropping large losses to the soil of total nitrogen occurred, without loss by leaching. The nitrogen withdrawn by the crops could not nearly account for the loss, so nitrogen must have escaped in gaseous form under these conditions. The nature of this nitrogen loss is still obscure. The
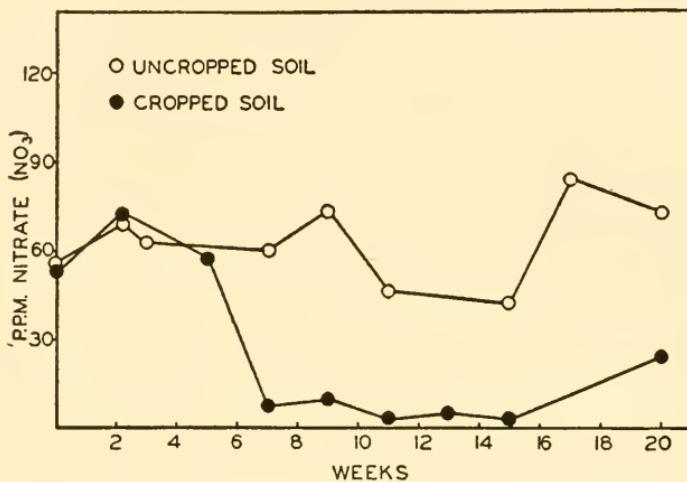
TEXTFIGURE 2. — Effect of cropping on nitrate present in soil solution at various periods of crop growth (barley crop). Soil solution nitrate concentration rises from low values in the spring or early summer, but with continued cropping of soils recovery of soil may not be adequate for requirements of a good crop. (From BURD, 1919).

soils were not subjected to an anaerobic environment, unless temporarily in local areas. Subsequently the total nitrogen levels have remained constant so far as can be determined. Whether or not a very slow further decrease is taking place, it is not possible to say.

Apart from supplying nitrogen to the plants, the nitrate ions formed from the reserve of organic mat-

ter are of signal importance in that the entrance of nitrate ions into the soil solution must of necessity be accompanied by the entrance of cations from the solid phase of the soil. Some of the soils also developed relatively high amounts of sulphate ions and in soils of the type studied bicarbonate ions likewise are significant anion components of the soil solution. The biological generation of anions, and of hydrogen ions, thus plays a dominant role in determining the concentration and composition of the soil solution, together with the reactions of soil colloids, now to receive consideration.

The Role of Soil Colloids: — In the early stages of the investigation of soil solutions there was lacking an essential key to an understanding of these phenomena; that is, adequate knowledge of the nature and behavior of clay colloids. Of greatest interest is the phenomenon of base exchange in colloids of this type. In the earlier period to which I have referred, ideas of base exchange were rather vague. Sometimes it was thought that base exchange was particularly a property of special minerals known as "zeolites." Then the systematic and valuable researches on soil colloids of the Russian chemist, GEDROIŽ, for a long time hidden in the Russian language, were translated into English and became familiar to American investigators, and the work of the Dutch investigator, HISSINK, also became known. The principle of stoichiometric replacement of adsorbed bases, primarily calcium, magnesium, potassium and sodium, and that of the large dependence of the physical properties of the colloid on the nature of adsorbed bases, were rapidly recognized to have profound consequences in soil science and plant nutrition. Interest in the subject of base exchange has increased, rather than diminished, in the years that have followed.

The significance of base exchange in clay colloids and of organic colloids also, is not difficult to appre-

ciate. A reserve of basic ions is held by the colloids, and these ions can in general be readily displaced by hydrogen ions biologically produced, from carbonic acid given off by roots or from acids formed by micro-biological processes, among which nitrification is of primary consequence. The group of California soils investigated were found to have their colloids saturated with calcium, magnesium and potassium mainly; calcium and magnesium predominated; of these two calcium was first in quantitative order. The solid phase of alkaline soils thus readily yields bases to the soil solution when hydrogen ions become available for ion exchange. But soils of humid regions are frequently acid. This is not merely a matter of a certain hydrogen ion concentration in the soil solution. Of deeper import is the fact that in highly acid soils much calcium and magnesium have been displaced from the colloids by hydrogen ions and then leached out of the soil.

There is a third type of soil condition with reference to the exchange status of the soil colloids and their interrelations with the soil solution which has had extraordinary importance in arid regions. I have in mind those soil conditions loosely grouped under the term "alkali" soils. These are soils in which large quantities of sodium salts are present, or have been present in the past history of the soil. If alkali soils are also highly alkaline, they are often referred to as "black alkali" soils, because of the accompaniment of a black crust of organic matter. About the origin of alkali conditions I can only mention that high water tables, or irrigation waters high in salt, are chiefly responsible. Frequently, the black alkali soils are very difficult to reclaim. On leaching out excess salt, which tends to maintain a flocculated state in the clay colloids, the soils soon tend to become highly impermeable to water. Indeed some soils of this kind once seemed to present a rather hopeless case. The real nature of the difficulty was not fully appreciated.

Later investigation based on the principles of base exchange made clear the dominating factors. In the black alkali soils the calcium held by the clay colloids had been replaced to a large extent by sodium, and

$$
\text{Colloid}\begin{array}{l}Ca\\Mg\\K\end{array} + \underbrace{HOH}_{\text{Water and}} + \underbrace{HHCO_3}_{\text{carbonic acid}} \rightarrow \text{Colloid}\begin{array}{l}H\\H\\H\\H\end{array} + \begin{array}{l}KOH\\Ca(HCO_3)_2\\Mg(HCO_3)_2\end{array} \qquad (27)
$$

Neutral clay   Acid clay   Removed from surface horizons by leaching

Ca Ca K Ca K

Na | Colloid | Mg

K Mg Ca Na Ca

Arid region
Desert soils
Arid brown soils
Chestnut soils

Ca Mg Ca K

Ca | Colloid | H
Mg | | H

Ca Na Ca

Transition zone
Chernozems

Ca H Ca Mg

H | Colloid | H
Mg | | H

Ca H Ca

Humid region
Gray-brown-podsolic soils
Podsols

Moisture region	Percentage composition of adsorbed cations					
	Na	K	Mg	Ca	H	Total
Arid (alkali soil).............	30	15	20	35	0	100
Transition (chernozem).......	2	7	14	73	1	100
Humid (podsol)..............	Trace	3	10	20	67	100

TEXTFIGURE 3. — To illustrate three general soil conditions and the relations of replaceable basic ions in each general type of soil. Note the different relative percentage of the several ions, especially of sodium, calcium and hydrogen ions. (From JENNY, Factors in Soil Formation, 1941. Courtesy McGraw-Hill Co.).

the sodium colloid has very different properties from the calcium colloid, properties which may lead to disastrous consequences for agriculture. To reclaim the soil it is necessary to displace and leach away sodium and to put back calcium in the colloid. The question

of how this can be done practically I shall not have time to explain. Dr. KELLEY has described in detail the experimental reclamation of an area of black alkali soil in the San Joaquin Valley of California. The point to be stressed is that researches on base exchange made the objective to be attained specific, and they also provided chemical tools for determining, from time to time, the effect of any given soil treatment. The significance of researches on alkali soils for the welfare of arid regions is not easily exaggerated. (*See plates 3, 4 and 5*).

The acid soil condition and the black alkali soil condition might seem to be entirely different phenomena. Considered from the point of view of base exchange, a fundamental unity is apparent. This is illustrated by citing a simple experiment made a long time ago by several of my colleagues, which was at the time enlightening. A markedly acid soil from a humid region was leached for some time with a sodium chloride solution and then with pure water. The acid soil became alkaline. Hydrogen ions of the original colloid had been displaced by sodium ions. While the soil was not converted into a typical black alkali soil in all respects, it did acquire some of the characteristics of the latter.

Enough has been said to show why the study of base exchange has been one of the strong trends in plant nutrition and soil science for two decades. Another trend in the study of soil colloids has appeared in recent years. Until this recent period the clay colloids were envisaged as merely amorphous bodies characterized by great development of surface and certain chemical properties of a weak colloidal acid. Some years ago HENDRICKS and FRY (1930) and KELLEY, DORE and BROWN (1931) began to make an application of the X-ray diffraction method to clay colloids. These investigators established that the clay colloids are capable of giving X-ray patterns betokening regular arrangement of atoms in crystal lattices.

The atoms are present as lattice ions. Occupying the larger part of the lattice space are the oxygen and hydroxyl ions, and in central positions lie smaller silicon and aluminum ions, or sometimes magnesium ions. The valence structure is balanced by adsorbed basic ions, particularly calcium, magnesium, potassium and sodium. Not only the extent, but also the kind of surface becomes important. Several general types of clay colloids have been distinguished on the basis of X-ray patterns and also of chemical and dehydration methods. The two most definitely characterized types are represented by montmorillonite and kaolinite. A third clay mineral related to the micas has been observed in some soil colloids.

To recognize the types of colloids occurring in various soils is both interesting and useful for many reasons bearing on chemical and physical properties of the soil. The kind of colloid may in part determine the relation between colloid and soil solution and so the fixation of chemical elements added to the soil in the form of fertilizers or soil amendments, a matter of high practical importance.

Soil Acidity and Alkalinity in Relation to Plant Growth: — In this limited survey I cannot pursue this subject into its various ramifications. I shall rather return to several other questions raised by the soil solution studies. One of these was that of soil acidity and alkalinity. At the beginning of the experiments in California, soil investigators and plant physiologists were not yet acquainted with physical-chemical concepts reflected in hydrogen ion or pH values, in their application to soil and plant systems. Many soils were described simply as "sour" soils. A rather common idea was that most plants of agricultural importance made their best growth in a slightly alkaline medium and that acidity was necessarily injurious. Sour or acid soils were often improved by liming, a fact long evident from agricultural practice.

The California soils with which we experimented were all slightly alkaline, according to general tests with indicators. With the introduction of the hydrogen

Si-Tetrahedron   Al-Octahedron

$\overset{\circ}{O}$ = (OH)
o = Al
• = Si
O = O

$\overset{\circ}{O}$ — (OH)
o — Al
• — Si
O —̣ O

7.3 Å.

15 ± Å.
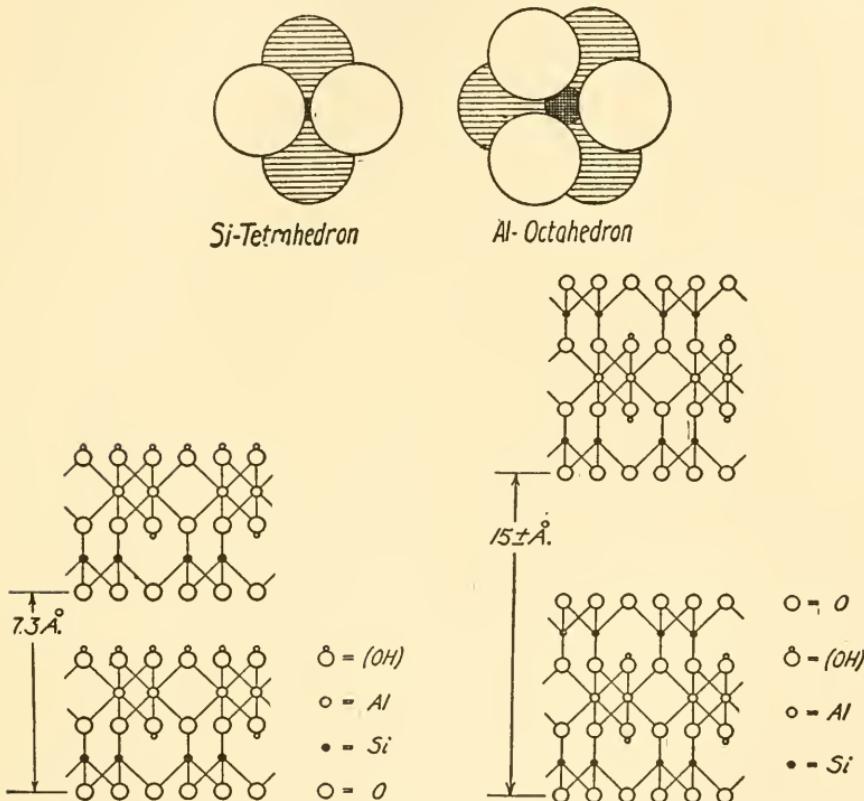
TEXTFIGURE 4. — *Above:* Two building units of crystalline structure of soil colloids. — *Below:* Showing proposed crystalline structures of two general types of soil colloids, kaolinite and montmorillonite. (From KELLEY, JENNY and BROWN, 1936).

electrode, the desirability of learning the effect of hydrogen ion concentration on plant growth at specific intensities of acidity or alkalinity became obvious. Was the alkaline reaction of the soils under study an indispensable requisite for high crop yields? In seeking an answer to this question plants were grown in

culture solutions in order to avoid the complexities of soil conditions. In view of impressions gained from the literature of the time, it was with some surprise that we observed that at a pH of 5 barley plants showed no injury and in fact this reaction was highly favorable. Later there followed in research in soil science and plant nutrition what might be called a hydrogen ion or pH period. Thousands of pH measurements on soils were made, and some enthusiasts sought to explain ecological distribution of plants and agricultural suitability of soils almost solely in terms of hydrogen ion effects.* But it has been learned that physiological relations of plants to their media are not so simple as that. Hydrogen ion concentration must be considered in relation to many other interreacting factors. It seems to be the internal reaction in plant cells rather than the external reaction that must be closely regulated. An opportunity will be afforded in subsequent lectures to say more on hydrogen ion relations of the culture medium to plant growth, but what I have already stated with reference to adsorbed calcium and hydrogen ions may be recalled now as pertinent to the general question.

Experiments by Artificial Culture Methods: — In addition to the hydrogen ion studies performed by the water culture technique, we inaugurated other water culture studies to ascertain if we could to some degree imitate soil solutions under more highly controlled conditions than a soil medium could afford. About this time a project was sponsored by the National Research Council, in which numerous investigators were invited to participate, for making water culture studies on plants with the objective of finding out the

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*The observation should also be offered that the interpretation of a hydrogen ion value on a complex system like that of the soil with its liquid and solid phases opens many questions. When measurements are made at low moisture contents, doubts may arise that the actually measured potentials really signify hydrogen ion concentrations or activities.

best proportions of nutrient salts in a culture medium. It was thought that this knowledge would have a practical application in guiding fertilizer practice. A considerable number of investigations were carried out. Several difficulties of technique and interpretation were involved, and it is well to examine one or two of these in the light of subsequent trends.

In the first place, plant physiologists at that period had given little attention to biological variability in plant culture experiments. An array of solutions of compositions varying by steps would be tested, and average values plotted on a triangular diagram, each axis representing one of the component salts of the nutrient solution. The attempt was made to assign small regions within the triangle as representing the best proportion of salts. One of my colleagues, A. R. DAVIS (1921), made an early experiment with a large number of replicate cultures of young wheat plants for each of several solutions and showed that, statistically evaluating the data, one could not single out within any narrow range a particular proportion of nutrient salts as representing a "best" solution. The results of the soil solution investigation that I have described were consistent with this conclusion. Several soils produced almost the same crop yields and yet the composition of the soil solutions differed markedly in ionic relations. Now the pendulum has swung in the opposite direction with regard to statistical evaluation of results in studies like these and the orthodox thing to do is to present an analysis by some statistical method. The mathematical analyses proposed by R. A. FISHER are most frequently employed.

Leaving aside the matter of statistical validity, there remained another difficulty in the water culture experiments. The solution placed in the culture vessel did not long remain the same, because of the absorption of salt by the plant. The solution was in fact continuously undergoing alteration. It was this ques-

tion of the nature of the process of absorption of salt by the plant that appealed to us as especially deserving of attention in the attempt to advance our understanding of the interrelations of soil and plant. I hope to return to these and related questions in later lectures.

In the early studies on soils and artificial nutrient solutions, almost always attention was centered on the seven classical essential elements that the plant had to obtain from its nutrient medium (nitrogen, calcium, magnesium, potassium, sulphur, phosphorus and iron). The essential role of other elements was unregarded, save by a few investigators, who themselves generally had no conclusive evidence. The development of knowledge of certain chemical elements needed by plants in minute amounts constitutes a striking feature of the study of plant nutrition, both practical and scientific, during the past decade. I intend to devote the next lecture to this subject.

Climatic Influences: — Finally, in all efforts to elucidate the relation of nutrient solutions to plant growth, the climatic complex enters into the equation. Experiments with culture solutions had all been made in greenhouses, subject to great fluctuations of temperature, light and humidity. Most experiments of necessity must be carried out in this way. In the modern period, however, various developments have occurred in the direction of efforts to impose for special experimental purposes as complete control as possible of the plant's environment; that is, control of the nutrient solution, illumination, temperature, humidity, and air movement. If I may mention only one of these developments for purposes of illustration, Dr. DAVIS and I, a considerable number of years ago, became interested in the use of air-conditioned chambers illuminated by artificial light, and succeeded in growing young wheat plants with such control of the environment of the solution and

of the air that according to available criteria the results in terms of plant yield and composition could be reproduced within small limits of error. Thus, a quantitative technique was established for various types of physiological studies.

In these experiments Mazda lights of high intensity furnished adequate illumination for the growth of wheat plants, even for the full cycle of development. But for most kinds of plants tested, the Mazda lamp illumination could not produce a satisfactory type of growth. The quality of the light was especially at fault. One recent trend in controlled chamber investigations is to employ various combinations of the new fluorescent lights. Quality of spectrum may be controlled to a considerable degree by combining fluorescent tubes of different colors, but often the problem of sufficient intensity remains unsolved, although useful studies may be made with the aid of these lamps. Some investigators with sufficient funds at their disposal have employed screened high-powered carbon arc lights. Since most experiments must be conducted in sunlight, the variability of the light factor is one of the outstanding difficulties in the quantitative evaluation of plant nutritional processes. (*See plates 6 and 7*).

Despite the importance of this consideration, even without the complete control of light and temperature environment, there still remain possible fruitful explorations into the effects of the aerial environment on the growth of plants in relation to their nutrition. This point may be illustrated by the experiments of THOMAS, HILL and their collaborators (1937). Ingenious apparatus has been constructed which makes possible the growth of plants in small glass houses with the automatic registration of CO_2 utilized in the sunlight, or produced in respiration during a dark period. The natural fluctuations in light intensity and temperature are measured at the same time. Control

of the root nutrient medium is exercised by growing the plants in sand culture through which nutrient solutions are passed at desired intervals. The adoption of technique of this kind affords many opportunities for examining the interrelations of light, temperature, and supply of inorganic nutrients. It would be of great interest to pursue this method of experimentation with the objective of elucidating further such questions as the influence of the nitrogen supply on the quality of the plant produced under a given climatic environment. The storage of sugar in the sugar beet may be cited as one example.

Physiological and Biochemical Investigations: — While I have stressed in this lecture the relation of inorganic solutes derived from the root medium to the growth of the plant, plant tissues are dominantly organic in nature when the water is driven off. The real problem of plant nutrition from the point of view of the plant, is not, strictly speaking, a problem of inorganic nutrition at all, but one of organic nutrition. What we should most like to learn about the inorganic nutrients is how, directly or indirectly, they enter into the synthesis and utilization of organic compounds. Thus far our knowledge of the functions of inorganic nutrients, except as they are present as components of the structure of indispensable organic compounds, is very scanty. Yet biochemistry in general has made great strides forward and the application of the principles and techniques of biochemistry to plant nutrition offers a path of progress.

Photosynthesis, of course, has received major attention, by eminent research workers in chemistry and physics, and this is an entire subject in itself. Aside from photosynthesis, there are many problems of plant biochemistry requiring long development. I might mention, for an illustration, the organic acid metabolism of plants because of its special interest in the study of the effects of inorganic nutrients on plant

metabolism. Only comparatively recently, however, has this organic acid metabolism begun to receive the attention it deserves. There are other phases of metabolism which likewise have a direct relation to the absorption of inorganic nutrients of which something will be said in later lectures. Then, too, we are all aware of the great advances made in the investigation of biological oxidation-reduction systems and their relations to certain metals required in plant growth in minute quantities. Still we have relatively little immediate evidence concerning these systems as they operate in higher plants. A large proportion of the accomplished research has been based on studies of animal tissues.

From the point of view of agriculture, as well as that of plant physiology, research workers have long been concerned with the relations of nitrogenous compounds elaborated in the plant to the assimilation of carbohydrate. Hormonal influences on flowering and on fruit development have recently been recognized to make this whole question much more complex than was at one time supposed. Nevertheless, as NIGHTINGALE has justifiably emphasized, the use of nitrogen and other soil nutrients by the plant has no realistic solution without comprehension of the chemical reactions that depend on interrelations between the inorganic nutrients and the products of photosynthesis.

In general I venture to say that biochemical attacks on plant nutrition will constitute one of the strongest trends of the future.

In this survey I have attempted to give you some impressions of the problems of the nutrition of higher plants and of some trends of research in a modern period. There are other trends of research in plant physiology that in some of their aspects have an indispensable relation to plant nutrition which I have not discussed, notably the development of the field of plant hormones. This, however, has been considered by your colleague, Dr. THIMANN and by others.

In concluding, I can only express the hope that I have established an introduction for the somewhat more specialized discussions to follow.

REFERENCES:-

BURD, JOHN S. Rate of absorption of soil constituents at successive stages of plant growth. Journal of Agricultural Research 18: 51-72, 1919.

—— —— and MARTIN, J. C. Water displacement of soils and the soil solution. Journal of Agricultural Science 13: 265-295, 1923.

—— —— —— —— . Secular and seasonal changes in the soil solution. Soil Science 18: 151-167, 1924.

CUMMINS, ARTHUR B. and KELLEY, WALTER P. The formation of sodium carbonate in soils. California Agricultural Experiment Station, Technical Paper 3: 1-35, 1923.

DAVIS, A. R. The variability of plants grown in water culture. Soil Science 11: 1-32, 1921.

—— —— and HOAGLAND, D. R. An apparatus for the growth of plants in a controlled environment. Plant Physiology 3: 277-292, 1928.

HENDRICKS, S. B. and FRY, W. H. The results of x-ray and microscopic examinations of soil colloids. Soil Science 29: 457-479, 1930.

HOAGLAND, D. R. The freezing-point method as an index of variation in the soil solution due to season and crop growth. Journal of Agricultural Research 12: 369-395, 1918.

JENNY, H. Factors of soil formation. McGraw-Hill, New York, 1941.

KELLEY, W. P. The reclamation of alkali soils. Calif. Agr. Exp. Sta. Bulletin 617, 1937.

—— —— and THOMAS, E. E. The removal of sodium carbonate from soils. Univ. of Calif. Agr. Exp. Sta. Technical Paper 1: 1-24, 1923.

—— —— and BROWN, S. MELVIN. Replaceable bases in soils. Calif. Agr. Exp. Sta. Technical Paper 15: 1-39, 1924.

—— ——, DORE, W. H. and BROWN, S. M. The nature of the base exchange material of bentonite, soils, and zeolites, as revealed by chemical investigation and x-ray analysis. Soil Science 31: 25-55, 1931.

—— ——, JENNY, HANS and BROWN, S. M. Hydration of minerals and soil colloids in relation to crystal structure. Soil Science 41: 259-274, 1936.

—— ——, WOODFORD, A. O., DORE, W. H. and BROWN, S. M. Comparative study of the colloids of a Cecil and Susquehana soil profile. Soil Science 47: 175-193, 1939.

PARKER, F. W. Methods of studying the composition and concentration of the soil solution. Soil Science 12: 209-232, 1921.

RICHARDS, L. A. A pressure membrane extraction apparatus for soil solution. Soil Science 51: 377-386, 1941.

STEWART, GUY R. Effect of season and crop growth in modifying the soil extract. Journal of Agricultural Research 12: 311-368, 1918.

THOMAS, MOYER D. and HILL, GEORGE R. The continuous measurement of photosynthesis, respiration and transpiration of alfalfa and wheat growing under field conditions. Plant Physiology 12: 285-307, 1937.

Lecture 2.

MICRONUTRIENT CHEMICAL ELEMENTS AND PLANT GROWTH

For about three quarters of a century the assumption was a general one by botanists and workers in the field of agricultural investigation that only ten chemical elements were universally indispensable for the growth of higher green plants; namely, carbon, hydrogen, oxygen, nitrogen, sulphur, potassium, calcium, magnesium, phosphorus and iron. The last seven were considered to be the essential elements of the nutrient medium. It was early recognized, of course, that many other chemical elements might be found in plant tissues, but this fact, while it gave a basis for certain presumptions, could not establish the essentiality of the elements present. Some investigators also observed that minute amounts of certain chemical elements added to the culture medium produced at times beneficial effects on plant growth. These were often regarded as "stimulating" effects. It was supposed that elements of a toxic nature could stimulate plant growth when present in extreme dilution.

MAZÉ in France (1914), following observations by other French workers, reported an investigation undertaken from a different point of view. He grew maize plants in highly purified salt solutions and concluded that chemical elements in rather large number were essential for the plant in very minute amounts. Important and fundamental in concept as MAZÉ's experi-

ments were, for most of the elements he considered essential the proof was not conclusive and only one species of plant was investigated, the corn plant. In any event, the work of MAZÉ received but scant attention for a long period of time. The teaching persisted that the plant's indispensable requirements were met by ten chemical elements, although it was often considered probable that plants of some species might have additional specific requirements.

In the early part of the twenties of this century extensive evidence was presented by MCHARGUE (1922) in Kentucky and by others that manganese must be regarded as an essential element for plant growth. Later experiments were carried out at the Rothamsted Experimental Station (BRENCHLEY and WARINGTON, 1927) originally for another purpose, that showed boron to be an essential element for broad bean plants. Interest was especially directed to the relation of boron deficiency to nodule development and nitrogen fixation by leguminous plants. This element was not at that time believed to be necessary for all species of plants. A boron requirement for barley, for example, was not demonstrated. During this same period SOMMER and LIPMAN (1926) in California inaugurated an investigation of essential elements, using highly refined technique. The culture solutions were made with salts especially purified for the purpose and with redistilled water, and other precautions were taken to avoid contaminations. With this technique it was possible to show that boron was a growth requirement for every kind of plant tested, including barley. It was not uniquely concerned with the growth of leguminous plants. Other experiments by LIPMAN and his associates yielded positive evidence of the essentiality of zinc for plant growth for a wide range of species. LIPMAN and MACKINNEY (1931) and SOMMER (1931) showed also that copper was essential. Thus there was a reason to add to the older list of

essential elements four more—boron, manganese, copper and zinc.*

So many investigators have now found it possible to demonstrate boron and manganese requirements for so many species of higher plants that these two elements are accepted, apparently, by all investigators. Some statements in the literature imply that not all are entirely ready to accept copper and zinc as generally indispensable elements. Yet in our laboratory we have never failed to show a need for these elements by any of the many species of plants subjected to rigorous test. It is well to recall that copper or zinc may appear unessential merely because enough of these elements gain entrance to the culture solution through impurities in reagents, in culture vessels or in the distilled water. Thus it is easy to fail to show a need for copper if ordinary laboratory distilled water is used in making the culture solution. (*See plates 8 and 9*).

Recent research in California by ARNON and STOUT (1939) gives strong evidence that another element will have to be added to the list of essential elements, namely, molybdenum. The need of this element by certain fungi had already been established. The list of essential elements is not closed. One can say about almost any chemical element that appears not to be essential only that it is not required in greater quantity than is represented by the unavoidable impurities in the culture solution. While not conclusively demonstrated to be essential over a wide range of species there are some

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*Elements in this category have been called by students of plant nutrition "rare" elements, "minor" elements or "trace" elements. All these terms seem to be inappropriate and I prefer as a general term "micronutrient" elements, fully realizing that objection can be found to this term also. Iron might logically be classified as a micronutrient element, but this is usually not done for the reason that this element has so long been recognized as a member of the list of essential elements along with those needed in larger quantities. A well illustrated book, "Hunger Signs in Crops", gives many examples of symptoms of deficiencies of these elements.

indications that silicon, aluminum and other elements may belong in the essential list. (*See plates 10 and 11*).

It is of interest to compare the needs of various types of organisms for micronutrient elements, to the limited extent present data make this possible. According to existing knowledge, animals (as represented by the rat, for example) have an essential requirement for manganese, zinc, and copper. Boron has usually been regarded as non-essential, but the difficulties of providing a boron free ration and the scarcity of investigation of this element from the point of view of the animal leaves the question open. The fungi carefully investigated, especially *Aspergillus* species, have requirements for zinc, copper, manganese, and molybdenum, but apparently none for boron. Another element, gallium, is assigned an essential role for these organisms by STEINBERG (1938). Molybdenum has a role in some nitrogen fixing bacteria. Iodine and cobalt, which have a function in animal metabolism, have not yet been shown to be indispensable for higher plants. Far more investigation is desirable of various lower organisms with reference to their requirements for micronutrient elements.

The quantities of boron, manganese, copper, zinc or molybdenum needed to insure good growth of a higher plant are extremely small, varying in concentration in a culture solution from less than .01 milligram in a liter to a few tenths of a milligram. In a culture solution, however, not only the initial concentration of the element, but also the total volume of solution supplied for a given number of plants requires attention, as well as interrelations among the micronutrient elements.

Functions of Micronutrient Elements: — The functions of these elements effective in minute amounts in plant growth and metabolism are still in a large measure obscure, although various suggestions can be made

based on physiological researches on plants or on the results of the biochemist, who often deals with animal tissues. The micronutrient elements must act as catalysts. That statement, of course, is too general to be of much value. We wish to know the nature of the reactions catalyzed. With regard to the metals, copper and manganese, as well as iron, something has been learned of the nature of oxidation — reduction systems in which those metals may be involved when associated with specific protein molecules — for example, poly- and monophenol oxidases, and ascorbic acid oxidase, in the case of copper. Certain researches also suggest that copper has a role in the synthesis of the porphyrin nucleus and thus in the formation of haem-compounds or of chlorophyll.

Manganese protein enzymes are not clearly defined, but there can be little doubt that manganese in plants does function in some oxidation system. LUNDEGÅRDH (1939) considers that manganese is of primary importance in the respiratory system of plants and BURSTRÖM (1938-39) has reported evidence that is interpreted to mean that the reduction of nitrate is dependent on an enzyme system in which manganese has an essential role. In some physiological experiments on barley plants in our laboratory by ARNON (1937) an interesting relation was disclosed between the form of nitrogen employed, either NH_4 or NO_3, the aeration of the culture solution, and the amount of manganese* furnished to the plants. In the solutions containing nitrogen as ammonium salt, increasing the manganese concentration, within certain limits, had a markedly beneficial effect, when the oxygen supplied to the roots was limited. Manganese also may have a role in the photosynthetic process, apart from the synthesis of chlorophyll. Experiments elsewhere on *Chlorella* suggested that increasing the supply of manganese increased the quantum efficiency but the interpretation

*Some effects were also found for copper and certain other elements capable of valence changes.

of such experiments has since been thoroughly revised. The possibility still seems to remain that manganese added to the culture solution, over the impurities usually present, may increase efficiency of photosynthesis.

No other micronutrient element has received so much attention as boron and still the mechanism of the function of this element in plant growth eludes us. Conceivably some clue will come from studies of the comparative biochemistry of different types of plants. As stated above, the fungus *Aspergillus* appears not to require boron and suggestively, perhaps, this organism has no essential need for calcium within the limits of positive demonstration by the techniques so far employed. A boron requirement on the part of *Chlorella* has not been shown although this may be merely a matter of inadequate technique. One idea concerning boron is that it has a role in the formation of pectin compounds. These contain galactose derivatives, which requires an inversion of H and OH on one of the carbon atoms, if they are formed from glucose. At any rate, boron does form compounds with some sugars or organic acids through adjacent OH groups and this property of boron needs consideration in further research.

Greatest emphasis has been given to possible interrelations between calcium and boron. Often there is a marked similarity or even practical identity of symptoms of calcium and boron deficiencies in plants. Both deficiencies are strikingly reflected in the failure of growth in meristematic regions of the plant. SHIVE (1941) and his co-workers have presented recently some evidence that boron in a still unknown way has a role in the determination of the state of calcium in the tissue, that is, the amount of calcium present in dissolved or easily soluble form. Several phases of organic metabolism need exploration before a good hypothesis for this effect of boron can be advanced.

Practical Aspects of Micronutrient Problems: — I
have been speaking of highly controlled experiments
with micronutrient elements. The elaborate precau-
tions necessary to prove that these elements are essen-
tial might at one time have made us highly sceptical
that deficiencies of chemical elements required in such
extremely small quantities would ever be manifested
under natural conditions in the field. It is now certain
that soils are not invariably capable of supplying
enough boron, zinc, copper, and manganese to main-
tain healthy growth of plants. This knowledge has
come mainly during the past ten years. Within this
period thousands of cases from many parts of the
world have been reported of crop failure or plant
disease resulting from deficiencies of micronutrient
elements. Boron deficiencies are surprisingly com-
mon in some regions. These statements do not imply
that most soils are deficient in any of these elements
but the areas involved are large and important enough
to warrant the view that the recognition of micro-
nutrient deficiencies constitutes a development in ap-
plied plant nutrition of major significance.

When I refer to deficiencies of boron, copper,
manganese, or zinc in the soil it is not a question of
absolute deficiency in total quantity of the element
present in the soil, but rather a physiological deficiency
arising from the insufficient availability of the element
to the plant; in other words, not enough of the element
can be absorbed and distributed in the plant for its
physiological needs at each successive phase of growth.
There are various possible reasons for the lack of
availability but it would take me too far afield to
discuss them, other than to mention presently certain
evidence pertaining to zinc and to add here that some
common agricultural practices may influence the ability
of the soil to supply micronutrient elements to the
plant. Boron deficiencies, for example, may in time
appear in a heavily limed soil.

Failure to recognize the essentiality of micronutrient elements in earlier periods led not only to misunderstanding of many diseases of plants growing in the field, but also to doubtful interpretations of supposedly well controlled experiments in greenhouses. When nutrient solutions were prepared without deliberate addition of micronutrient elements, there was no assurance that fully adequate amounts of these would be accessible to the plant, even though its demands were very small. Some lots of nutrient salts were probably freer from impurities than other lots, and contaminations from culture vessels and from distilled water varied in unknown degrees. Consequently, the possibility was not excluded that some of the effects of the culture solutions on plant growth attributed mainly to elements in the classical list, did in fact have a relation to the uncontrolled micronutrient elements. Some specific instances could be mentioned to show that the earlier investigations of plant nutrition by artificial culture methods were subject to these complications.

There is another aspect of the physiology of one of the micronutrient elements of marked agricultural interest. Boron, essential as it is, can become toxic when present in the nutrient medium of plants in concentrations not much higher than those which are favorable. In some parts of California irrigation waters contain enough boron so that it accumulates in the soil to a point of injury to sensitive crops. (KELLEY and BROWN, 1928; EATON, 1935). The boron toxicity problem has been extensively investigated in a Federal laboratory at Riverside established for the purpose. Many controlled sand culture experiments were made which had not only practical but also physiological interest. Different species of plants differ most significantly in their ability to absorb boron from a given medium and in susceptibility to injury. Some citrus species are very sensitive; alfalfa tolerant. In citrus large amounts of boron can accumulate in
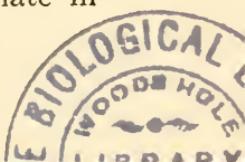
the leaves while this is not true of some deciduous fruit tree species. Work on boron, from the point of view of both deficiency and toxicity, constitutes a development in the study of plant nutrition of important consequence to agriculture in certain regions.

Discussions of Zinc as a Micronutrient Element: — With this general survey before you, I wish to devote the rest of my time to the discussion of one micro-nutrient element that has attracted great interest in California and elsewhere — the element zinc. By describing researches on this element, I think that I shall be able to illustrate the general nature of the micro-nutrient problem in its various aspects, scientific and agricultural.

Somewhat more than ten years ago an investigation was begun in California of a disease of deciduous fruit trees, known as the "little-leaf" disease. In the particular peach orchard first studied, located on a sandy soil, the trees had made remarkably good growth for several years — then they became affected with the "little-leaf" disease, as it was often called.* No ordinary fertilizer treatment had any beneficial effect and plant pathologists could find no evidence that pathogenic organisms were primarily responsible for the condition of the trees. A micronutrient deficiency was early suspected but the treatment at first applied to test this possibility was not followed by any ben-eficial result. Another special treatment of the soil consisting of a large application of commercial iron sulphate, used for another reason, was successful. Further experimentation showed that the reason why this treatment succeeded was that the iron sulphate contained a considerable amount of zinc as an im-purity and that the properties of the iron sulphate had an influence in preventing too rapid fixation of zinc in the soil.

*For a more complete review, *see* W. H. CHANDLER, 1937.

Experiments were extended by other California investigators to citrus orchards, in which the trees showed the symptoms of the disease known as "mottle-leaf," a disease of frequent occurrence in California. This disease had been studied for about twenty years without any good clue as to its cause. Many hypotheses had been advanced but no one of them was consistent with all the observed facts. It is now clear that the mottle-leaf disease is also caused by zinc deficiency. There are practical difficulties in correcting this condition by addition of zinc salts to the soil, but spraying the trees with appropriate zinc compounds is effective and is at present a common commercial practice. Zinc deficiencies in field grown crops are now known in many parts of the world. They often occur in Florida and other southern states, from which independent evidence became available of zinc deficiency as the cause of certain nutritional diseases of crops, for example, pecan "rosette". In Australia a disease of pine trees has been traced to zinc deficiency. In Hawaii pineapple plants grown in certain soils produce abnormal, distorted blades, recently recognized to be the result of zinc deficiency. The condition is easily remedied by zinc sprays. (*See plate 12*).

When a plant growing in soil suffers from lack of zinc, obviously this means that the plant cannot absorb enough zinc for its needs. Yet the total amount of zinc present in the soil may be ample. To illustrate, in the California peach orchard to which I have referred, the trees became severely diseased from lack of zinc and nevertheless, the mass of soil within the root zone contained enough total zinc to meet the requirements of the trees for many centuries. The reasons for unavailability of zinc in such cases may be various, and include several types of chemical fixation. In some of these the zinc is fixed in the crystal structure of soil colloids. It is also of interest to suggest the possibility that soil microorganisms sometimes

have a role in determining zinc availability to crops. In our greenhouse experiments with a soil producing zinc deficiency disease in corn (and in the field the "little-leaf" disease of peach trees) we have found that frequently this failure of the soil to supply zinc can be overcome by sterilizing the soil and that the condition of deficiency in supplying power for zinc can be reestablished by reinoculating the sterilized soil with a very small percentage of unsterilized soil. One assumption is that soil microorganisms growing close to or in contact with root surfaces offer competition for minute amounts of zinc present in dissolved or available form*. (*See plate 13*).

The interest of a biologist will be attracted by the great differences among different species of plants in their ability to absorb zinc from a soil with low zinc supplying power. In the greenhouse alfalfa has been observed to obtain sufficient zinc from a soil in which corn plants suffer severely from lack of zinc. Sometimes the continued growth of alfalfa in an orchard will prevent the development of little-leaf disease in trees. By solution culture technique, however, it is possible to show that alfalfa itself has a zinc requirement not very different in magnitude from that of other plants that fail for lack of zinc in the soil in which alfalfa grows satisfactorily. Whether alfalfa roots have a special capacity for absorbing zinc ions or whether the nature of the soil flora undergoes change as a result of the crop growth with an accompanying influence on the available zinc status of the soil remains unsettled.

While the applied agricultural aspects of zinc deficiency have a high degree of importance for a member of an Agricultural Experiment Station, I appreciate that this audience would be far more in-

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*These experiments have been frequently repeated and in different years. However, failures have occurred. These are perhaps to be expected, since under greenhouse conditions soils after sterilization may readily become accidentally contaminated with various microorganisms.

terested in having me trace definitely the function of zinc in living organisms. Unfortunately that cannot as yet be done, but I should like to report a few physiological and biochemical experiments that possibly have some value as starting points for further research. The difficulty confronting us is to fix the role of zinc in any of the chains of processes that result in plant growth as an integrated effect. A missing link anywhere in any one of the chains will bring growth failure or deranged metabolism. That difficulty of course is not peculiar to the study of zinc.

The quantitative requirement of a plant for zinc is not simply determined. It is in part governed by climatic factors. We have made experiments in a greenhouse at various seasons of the year with corn plants growing in solutions containing graduated amounts of zinc. In the winter some retardation of growth, but only slight leaf symptoms of zinc deficiency appeared, even on plants in the highly purified solutions containing only extremely small amounts of zinc. In similar solutions the plants would die in summer during periods of high illumination (STOUT and HOAGLAND, unpublished). These seasonal effects were manifested also in experiments with a zinc deficient soil. The symptoms of zinc deficiency shown by plants grown in summer could be decreased in severity by shading the plants with cheese cloth. Likewise under conditions in the field climatic effects seem to be important. Generally the zinc deficiency diseases are found in regions of high summer light and temperatures; in California, not often in the foggy coastal belt. With citrus the observation has been made by several investigators that the sunny side of the tree is usually much more mottled than the shady side, if zinc deficiency prevails in the soil. (*See plate 14a and b*).

Some further information on this question was secured incidental to a study of the distribution of zinc in different parts of the plant by P. R. STOUT.

Tomato plants were set out in a nutrient solution very low in zinc and at the season when the experiments were made the plants were subjected to good natural illumination.   Marked failure of the plants to continue growth was observed.   The plants were then brought to the laboratory with its less intense light. Without any further addition of zinc the plants resumed growth.

It happened that this was an experiment in which radioactive zinc had been supplied to the plants in extremely small amounts.   It was therefore possible to show that the zinc originally present in the stems was translocated to growing points under the low light conditions of the laboratory.   The explanation of this response may be that a breakdown of zinc protein compound in one part of the plant occurs under reduced light, with a release of zinc and its transportation to a region capable of more active growth.

I wish to turn now to another series of experiments in which a light factor associated with zinc nutrition appears to be involved.   Sometime ago we conceived the idea, based on various observations unnecessary to describe now, that there might exist some sort of interrelation between zinc and an auxin growth substance.   At that time Dr. SKOOG, recently a member of your staff, came to Berkeley and thought it worthwhile to pursue the suggestion further.   I shall have time to indicate only the general nature of some of his results (SKOOG, 1940).   When tomato plants were grown in zinc deficient solutions in full greenhouse light the plants failed to elongate and their auxin content was extremely low.   Addition of a small amount of zinc to the culture solution soon caused a large increase of auxin in the plant and subsequently elongation took place.   When the plants were placed under a red light filter of cellophane the same zinc deficient solutions permitted considerable elongation of the stem and its auxin content was higher than that of the zinc deficient plants subjected

to normal illumination. It may be added that short
wave light seems to increase the rate of breakdown of
auxin.

The low zinc plants with a low auxin content had
a higher peroxidase activity than that of the plants
with a larger zinc supply and Dr. Skoog postulated in
accordance with the view originally proposed at the
California Institute of Technology on the basis of
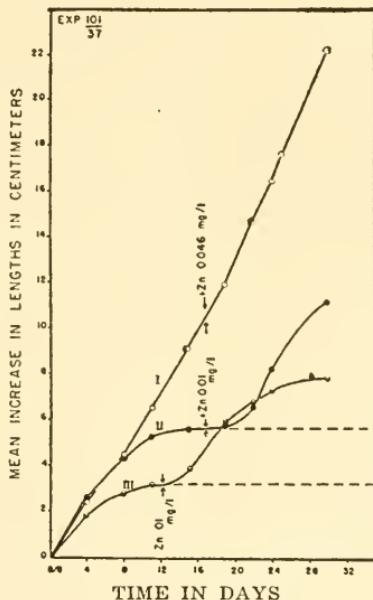
TEXTFIGURE 5. — Tomato plants grown in
solutions with and without an adequate supply
of zinc. Response in stem elongation follow-
ing addition of zinc to a zinc deficient culture
is clearly evident. Curve I, plants originally
supplied with 0.046 mg. per liter Zn; Curve
II, 0.01 mg. Zn; Curve III, no added Zn.
(From Skoog, 1940).

other types of experiments, that the destruction of
auxin was accelerated by the increase of peroxidase
activity. Later BEAN in our laboratory made further
experiments relating to this question and also found
a relation between zinc and the peroxidase and catalase
activities of the plant tissue.

These complicated interrelations of auxin and zinc in terms of elongation by no means explain all the responses of the plant to zinc deficiency. For example, REED and DUFRÉNOY (1942) have shown that profound cytological changes occur in green leaf cells and in growing points as an end result of zinc deficiency. In the leaves of certain species of fruit trees the presence or the form of phenolic substances in cell
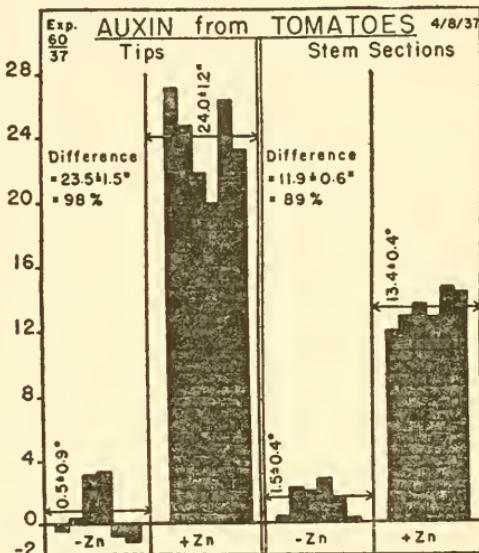
TEXTFIGURE 6. — Continued from textfig. 5. (From SKOOG, 1940).

vacuoles was a general character accompanying the disease, although these effects are not necessarily specific to zinc deficiency. Destruction of chloroplasts or inhibition of their formation is generally noted in examination of leaf cells of zinc deficient plants. Lytic factors may destroy most of the cell contents in extensive areas of tissue. (*See plate 15*).

Protein synthesis in the plant is markedly influenced through some direct or indirect action of zinc, for which view I may cite briefly some recent experiments conducted in our laboratory by BEAN (1942).

The experimental plant was again that guinea pig of the plant physiologist—the tomato. Clearly not much can be learned about the function in a plant of a nutrient element if the deficiency is so acute that the plant makes almost no development. In these experiments the supply of zinc was so regulated for some sets of plants that the plants attained only an incipient state of zinc deficiency; that is, there was no
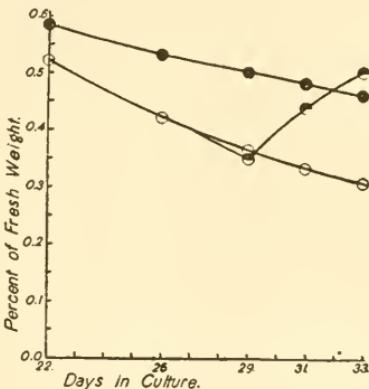
Protein nitrogen in leaf blades of Experiment B, calculated as the difference between total nitrogen and total soluble nitrogen. ● High-zinc series. ○ Low-zinc series. ◐ Recovery series.

TEXTFIGURE 7. — Protein nitrogen in leaf blades of tomato plants as influenced by application of zinc during growth, showing response in protein synthesis in zinc deficient plants after adding zinc. The deficient plants were in only an incipient state of zinc deficiency. (From R. S. BEAN, 1942).

observable symptom of deficiency within the time period allowed, although it was known that zinc deficiency symptoms would appear later. Other sets of plants had a larger supply of zinc, sufficient to protect them against zinc deficiency for a long period. At

the time when the plants were considered to be at a suitable stage of growth samples of leaf tissue were taken from low and high zinc plants.

One of the indications from the experiments was that the incipient zinc deficiency resulted in a marked effect in retarding protein synthesis. Especially interesting was the rapid resumption of protein synthesis when zinc was supplied to the low zinc plants. Starch synthesis was likewise retarded by zinc deficiency although the sugar content of the plant was not diminished. This is in contrast to the effects of boron deficiency. With this deficiency the tomato plant may greatly increase its percentage content of both sugar and starch.

Again in a speculative vein one might attempt to explain these responses in protein and starch synthesis on the assumption that zinc is a component of a catalytic system necessary for the phosphorylation of glucose (known to be a step in starch synthesis) or possibly of an amino acid. We must acknowledge, however, that the only secure proof of the function of an element like zinc is to isolate an enzyme system for the operation of which the element is essential. So far as I am aware there is evidence of this kind for only one reaction in which zinc is concerned. KEILIN and MANN (1939) have reported the isolation of a zinc protein system (from blood corpuscles) that catalyzes the reversible reaction of $H_2CO_3 \rightleftarrows CO_2 + H_2O$. It is conceivable that a similar enzyme system may function in respiratory or photosynthetic processes of the plant, but adequate proof of this is lacking.

As one other observation, REED has demonstrated that partial zinc deficiency has special effects in inhibiting seed formation or development.

The possibility exists, according to the views of REED and DUFRÉNOY, that deficiency of zinc may bring about a disturbance in oxidation-reduction systems in the plant. Zinc does not undergo reversible valence changes, so any function it may have in oxidation-

reduction systems must be less direct. In general, one could conceive that the various metals do not act entirely independently. If they have a role in protein enzyme systems, the metals might compete for reactive groups in the protein molecule, although enzyme activity would be dependent on specific metals. I have already referred to the effect of zinc deficiency on the oxidase and catalase activities of the leaves of tomato plants. It may also be recalled that after increasing the concentration of zinc in a culture solution beyond a certain point, a leaf injury or chlorosis may follow, that suggests an iron deficiency. Another case in point is that of interrelations between iron and manganese.

Minute Amounts of Chemical Elements in Relation to Animal Nutrition: — Before closing I wish to draw your attention to an aspect of the investigation of micronutrients that concerns students of both plant and animal nutrition. The animal depends in part for its supply of inorganic elements effective in minute amount on the plant. What the plant absorbs from its medium is therefore of consequence to the nutrition of the animal consuming the plant. Some micronutrient elements needed by plants are also needed by animals. This seems to be true of copper, zinc and manganese. About boron we are uncertain, as I have said.

A large amount of evidence supports the conclusion that animals in certain areas can suffer from deficiency diseases because the vegetation consumed does not supply enough of one or more of the elements that function in minute quantities. The requirements of the plant and of the animal are not necessarily coincidental quantitatively, and perhaps not always qualitatively. The element cobalt furnishes an illustration of the latter statement. A number of years ago the discovery was made that in some districts in New Zealand and Australia, sheep suffered from a

disease of some apparently nutritional character. At first, it was supposed that there was a deficiency of iron in the ration. Actually, later experience showed that primarily the trouble was a deficiency of cobalt, small as the amount required was. The pasture plants upon which the animals fed did not seem to give any indication of deficiency of cobalt but they did not contain enough cobalt for the needs of the animal. We have not yet had reason to think that cobalt is an essential element for higher plants. But under the field conditions I have described there exists a question of availability of cobalt in the soil and of the absorption of cobalt by the plant, as far as the welfare of the grazing animal is concerned.

The problem of animal nutrition in relation to the physiology of the plant and to soil conditions is not restricted to deficiencies of chemical elements. Plants may become toxic to the animal because of something the plant has absorbed from the soil. Doubtless in this connection you are familiar with the example of selenium, since so much recent discussion of this element has appeared in both scientific and popular journals. It will be recalled that an explanation was found of a previously obscure disease of cattle, horses and other animals occurring in South Dakota, Wyoming and elsewhere, which had been called sometimes the "alkali" disease. Now it is clear that the animals suffering from this disease are poisoned by selenium derived from the vegetation growing on certain types of soil. The soils supporting this vegetation are derived from shales high in selenium.

Of particular interest to this discussion is the selective power of different plant species in the absorption of selenium from the same soil. Some plants take up relatively little of this element, while others accumulate large amounts. In the latter class are several species of *Astragalus*. When the residues of high selenium plants are returned to the soil and undergo decomposition, selenium is left in a more avail-

able form for other plants than was originally present. Plants of this kind are at times called selenium "converters." Experiments have been made on certain *Astragalus* species to ascertain if the plant itself benefits from the absorption of selenium. Apparently some species are favored in their growth by the presence of selenium in the medium. The suggestion has even been made that selenium is possibly an essential element for some species (TRELEASE and TRELEASE, 1938).

One more case of lesser importance may be mentioned of the absorption by the plant of a chemical element to the point of toxicity to animals. The report has come from England that in some soils plants absorb enough molybdenum to raise the plant's content of that element sufficiently to produce a pathological condition in the animals consuming the vegetation grown on these soils.

I should like to emphasize the interest inherent in the broad question of the interrelation of soil, plant and animal, which is of course not limited to the micronutrient elements. Other inorganic plant nutrients and also organic substances synthesized by the plant are involved. The importance of this field of study has been considered by the Federal government great enough to warrant the establishment of a new laboratory at Cornell University with the specific objective of investigating the factors that govern the quality of plant products from the point of view of human and animal nutrition as distinguished from quantitative yield. This is a practical objective, but in the course of these and other researches of similar trend, we may expect that our views of the nutrition of both plant and animal will be widened, and that we shall gradually perceive many unifying principles of metabolism common to living organisms of different categories. The micronutrient elements present one interesting aspect of these convergent investigations.

REFERENCES:-

American Society of Agronomy and American Fertilizer Association. Hunger signs in crops. 1941.

ARNON, D. I. Ammonium and nitrate nitrogen nutrition of barley at different seasons in relation to hydrogen ion concentrations. Soil Science 44: 91-120, 1937.

—— ——. Micro elements in culture-solution experiments with higher plants. American Journal of Botany 25: 322-325, 1938.

—— —— and STOUT, P. R. Molybdenum as an essential element for higher plants. Plant Physiology 14: 599-602, 1939.

AUCHTER, E. C. The interrelation of soils and plant, animal and human nutrition. Science 89: 421-427, 1939.

BEAN, ROSS SMOOT. The effect of zinc on nitrogen metabolism and on certain oxidizing enzymes in leaves of the tomato plant. Thesis for Ph.D. degree, University of California, 1942.

BRENCHLEY, WINIFRED E. The essential nature of certain minor elements for plant growth. Botanical Review 2: 173-196, 1936.

—— —— and WARINGTON, KATHERINE. The role of boron in the growth of plants. Annals of Botany 41: 167-187, 1927.

BURSTRÖM, H. Über die Schwermetallkatalyse der Nitrat-Assimilation. Planta, Archiv für Wissenschaftliche Botanik 29: 292-305, 1938-39.

CHANDLER, W. H. Zinc as a nutrient for plants. Botanical Gazette 48: 625-646, 1937.

EATON, FRANK M. Boron in soils and irrigation waters and its effects on plants, with particular reference to the San Joaquin valley of California. U. S. Dept. Agr. Tech. Bull. 448, 1935.

HOAGLAND, D. R. Water culture experiments on molybdenum and copper deficiencies of fruit trees. Proc. Amer. Soc. Hort. Sci. 38: 7-12, 1940.

—— ——, CHANDLER, W. H. and HIBBARD, P. L. Little leaf or rosette of fruit trees, V. Effect of zinc on growth of plants of various types in controlled soil and water culture experiments. Proc. Amer. Soc. Hort. Sci. 33: 131-141, 1936.

KEILIN, D. and MANN, T. Carbonic anhydrase. Nature 144: 442-443, 1939.

KELLEY, W. P. and BROWN, S. M. Boron in the soils and irrigation waters of southern California and its relation to citrus and walnut culture. Hilgardia 3: 445-458, 1928.

LIPMAN, C. B. and MACKINNEY, G. Proof of the essential nature of copper for higher green plants. Plant Physiology 6: 593-599, 1931.

LUNDEGÅRDH, H. Mangan als Katalysator der Pflanzenatmung. Planta 29: 419-426, 1939.

MCHARGUE, J. S. The role of manganese in plants. Journal American Chemical Society 44: 1592-1598, 1922.

MAZÉ, P. Influences respectives des éléments de la solution minérale sur le développement du maïs. Ann. Inst. Pasteur 28: 21-68, 1914.

REED, HOWARD S. Cytology of leaves affected with little leaf. American Journal of Botany 25: 174-186, 1938.

—— ——. Effects of zinc deficiency on cells of vegetative buds. American Journal of Botany 28: 10-17, 1941.

—— —— and DUFRÉNOY, JEAN. Catechol aggregates in the vacuoles of cells of zinc deficient plants. American Journal of Botany 29: 544-551, 1942.

SHIVE, JOHN W. Significant roles of trace elements in the nutrition of plants. Plant Physiology 16: 435-445, 1941.

SKOOG, FOLKE. Relationships between zinc and auxin in the growth of higher plants. American Journal of Botany 27: 939-951, 1940.

SOMMER, A. L. Copper as an essential for plant growth. Plant Physiology 6: 339-345, 1931.

—— —— and LIPMAN, C. B. Evidence on the indispensable nature of zinc and boron for higher green plants. Plant Physiology 1: 231-249, 1926.

STEINBERG, ROBERT A. The essentiality of gallium to growth and reproduction of *Aspergillus niger*. Journal Agr. Res. 57: 569-574, 1938.

STOUT, P. R. and ARNON, D. I. Experimental methods for the study of the role of copper, manganese, and zinc in the nutrition of higher plants. American Journal of Botany 26: 144-149, 1939.

TRELEASE, SAM F. and MARTIN, ALAN L. Plants made poison-ous by selenium absorbed from the soil. Botanical Review 2: 373-396, 1936.

—— —— and TRELEASE, HELEN M. Selenium as a stimulating and possibly essential element for indicator plants. Ameri-can Journal of Botany 25: 372-380, 1938.

WARINGTON, KATHERINE. The effect of boric acid and borax on the broad bean and certain other plants. Annals of Botany 37: 629-672, 1932.

THE ABSORPTION AND ACCUMULATION OF SALTS BY PLANT CELLS

In the last lecture I undertook to discuss the chemical elements derived from the plant's root medium that are indispensable to the growth of the plant. Manifestly this subject constitutes a fundamental aspect of plant nutrition, but not less fundamental are problems of the entry into the roots of these essential chemical elements and of their upward movement and distribution in the plant. These are the problems I shall deal with in this and the following lecture.

Interest in this general field of research by the laboratory with which I am associated was initially awakened during the first world war when we became engaged in a comprehensive investigation of the giant kelps of the Pacific Coast, with intent to gain knowledge that would be helpful in recovering the then much needed potash present in the kelps. We were impressed by the remarkable selective accumulation by these plants of potassium, iodide and bromide and by the lack of satisfactory knowledge concerning the physiological processes that led to the absorption of these ions from the sea water and their differential retention in the plant tissues, apparently largely in inorganic form. The undertaking of special research on this problem came later, however, and was more immediately stimulated by the many questions that arose from the soil researches described in the first lecture. It was evident that these questions could

never be satisfactorily approached if experiments were limited to plants growing in so complex and so diffi- cultly controllable a medium as that of the soil. There- fore, experiments on barley plants growing in liquid media — by the so-called water culture method — were initiated and several general relations between the composition of the nutrient media and the absorption of nutrient ions by the plant were observed.

Even at this earlier period a relatively vast litera- ture existed on the subject of permeability of plant and animal cells and certain concepts of antagonism of ions had been well developed. Most of the methods employed in the study of permeability were indirect and frequently utilized conditions under which the normal functioning of the cell could not be expected to continue. Many studies were made on marine or- ganisms living in media of high concentration and so the conclusions were applied to plants growing in soils with reservations. Specifically, we found compara- tively little enlightenment from most of the permea- bility researches with regard to the nature of the processes occurring when plants grow in dilute salt solutions comparable to those of many soil solutions. It did not appear from our results, or from other per- tinent evidence, that the intake of the nutrient ions was merely a diffusion process proceeding to attain- ment of equal concentrations or activities of a solute in internal and external phases, as many texts then, as well as some much more recent texts, taught.

Studies on Nitella Cells: — In the state of develop- ment of the subject at that time there were more doubts than now concerning the interpretation of evi- dence from experiments on complex plant tissues, especially when the data were obtained by analyzing the whole tissue or the saps expressed from the tissue. Temporarily, therefore, we later turned to the fresh water alga *Nitella,* which produces multinucleate inter- nodal cells, often several inches in length, from which

sap largely uncontaminated by other components of the cell can be recovered (HOAGLAND and DAVIS, 1929). Thus it is possible to make definite comparisons of internal and external fluids separated by a cell wall and layer of protoplasm, to reduce this system to the simplest terms.

A few semi-quantitative analyses of sap had been made on large marine cells — the *Valonia* cells, which subsequently were to receive so much attention — but the *Nitella* cells were of special interest for our pur- pose because the pond water in which they lived and from which they absorbed salt was after all not funda- mentally different from some soil solutions in total concentration of electrolytes. The analyses of *Nitella* sap and pond water indicated quite clearly and un- ambiguously that the cells must have absorbed all the principal ions they contained against concentration and activity gradients. The cells obviously had ab- sorbed salt during their development, but from one point of view they were highly impermeable to salt, since they could be placed in distilled water and unless they were injured or their metabolism damaged, prac- tically no salt was lost to the water from the vacuoles. (*See plate 16*).

The vacuolar sap was found to be primarily a salt solution and the ions were neither held to an ap- preciable extent in an adsorbed state nor precipitated out in the form of insoluble compounds. The electrical conductivity of the sap was about what would be expected on the basis of the total salt in the sap if this was present in ionized form. The distribution of ions could not be explained in its major aspects in terms of the Donnan equilibrium, which comes into play when an ion of one sign of charge cannot pass through a membrane. After the general relation of the ionic composition of the vacuolar sap of the cells to that of the pond water medium became established it was decided to try experimental procedures to deter- mine whether or not additional amounts of ions could

be caused to enter the cells. For this purpose a test ion not already present in the cell was desirable. The bromide ion was found to be well suited to the need. It could be determined with some facility and was not toxic.*
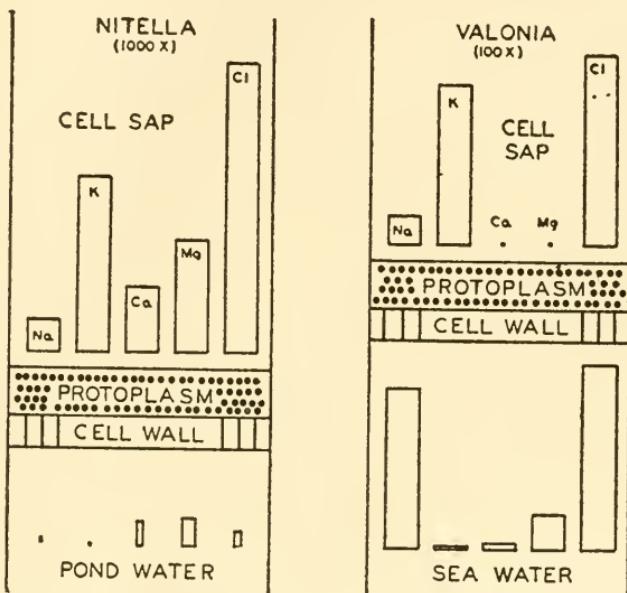
TEXTFIGURE 8. — Diagrammatic representation of relative concentrations of several ions in the culture medium and in the vacuolar sap of *Nitella* and *Valonia* cells. All ions shown (and PO$_4$ions also) reach a much higher concentration in the sap of *Nitella* than in the external solution. In *Valonia* potassium is concentrated, but not sodium, calcium or magnesium. Sea water is primarily a solution of sodium chloride and the cell sap of potassium chloride.

The experiments with bromide and some in which potassium also was studied showed that the large *Nitella* cells retained a limited capacity to take in more ions against gradients, but it seemed to be a necessary conclusion that this capacity was dependent on meta-

*Subsequently the bromide ion has served a valuable purpose in many investigations of the accumulation and transport of ions in higher plants.

bolic activities of the cell by which cellular energy was made available for ion transport. Effects of light and temperature on the process of ion accumulation were in accord with this conclusion. Another interesting and significant fact was that the *Nitella* cells, as long as they remained uninjured, maintained an approximately constant hydrogen ion concentration in the vacuolar sap at the value of pH 5.2, even when the outer medium varied in its reaction over so wide a range as nearly 5 pH units. At the same time this sap, chemically considered, was not highly buffered.

I shall not dwell on these early studies on *Nitella,* useful as they were at the time they were made. Rather I should like to interject here a brief discussion of some recent experiments on *Nitella* cells. After many years investigators are in a position to return to research on large cells of this kind with the aid of radioactive isotopes as tracers. I believe that my colleague, Dr. S. C. Brooks, was the first to utilize isotopes in experiments on *Nitella* cells. He has carried on studies especially with radioactive rubidium (Brooks, 1940). In these studies a rough separation was made of the cell wall, the protoplasm, and the vacuolar sap so that these several parts of the cell system could receive separate examination. The rubidium was found to enter the protoplasmic phase extremely rapidly and reached there a high net concentration, much higher than that of the external solution. Entry into the vacuole was, however, very slow and during the experimental periods the concentration of rubidium in the vacuole did not reach nearly so high a value as that of the protoplasm.

Mr. Broyer and I (1942) have also made experiments on *Nitella* with radioactive isotopes, from the point of view of our own problems, especially as they are related to the investigation of salt absorption by roots, which I shall discuss presently. We have found that radioactive bromide can accumulate at first in the protoplasmic phase of *Nitella* cells, but subsequently

there is continued movement of ions into the vacuole, until the concentration there seems eventually to become higher than in the protoplasm and certainly higher than that of the external dilute solution.*

The accumulation of bromide in the vacuolar sap takes place when the cells are in the light and so evolving oxygen; also in the dark, at least for a limited period, if aeration is provided. In the experiments so far performed the *Nitella* cells did not accumulate in the vacuolar sap either bromide or rubidium to a concentration higher than that of the external solution, except when the cells were in an aerobic environment. There is an indication from these experiments of the operation of a process akin to secretion and this point will be of continued interest as our discussion proceeds. I may add here that Dr. BLINKS of Stanford University conducted experiments with radioactive rubidium on large marine *Halicystis* cells. The general conclusions are similar to those based on work with *Nitella* cells.

Use of Radioactive Isotopes: — This may be the place to interrupt the discussion in order to make a general observation on the use of radioactive isotopes, since I shall report experiments in other lectures in which these isotopes were found useful. It might first be asked whether or not the radiation of the exploding atoms injures the cells. Although this might happen we are satisfied that this factor does not invalidate evidence from experiments of the kind reported. The radiation given off is too weak. Barley plants have

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*In these experiments measurements were actually made on the cell vacuolar sap and on the residue of the cells after removing the sap. BROOKS had observed that very little of the radioactivity of the *Nitella* cells could be assigned to the cell wall. In the present experiments values were computed in terms of the total moisture of the residues and the computed concentrations for protoplasm may therefore be low. Nevertheless, it is significant that with the elapse of time relative concentrations of the test ions in the sap increase more than those in the residue.

been grown for a long time in solutions of radioactive phosphate of much higher activity than that regularly employed, without any perceptible injury to the plant.

In the field of plant physiology we regarded the radioactive isotopes to be of particular value as an additional tool of unique kind for an investigation already established. We have not devised any in- vestigation to fit the tool. Often the isotopes are em- ployed for convenience; the same things might some- times be done in other ways, although perhaps with far more difficulty. There are, however, certain facts to be learned only by the isotope method. For ex- ample, it may be shown with roots that it is possible for ions to move out of a tissue while other ions of the same species are moving in more rapidly, with the result that there is a net gain of the ions in the tissue. Further, the radioactive elements can make their own pictures and thus demonstrate directly their general distribution in the plant. They can indicate their presence in some cases without any operation on the plant, by application of a Geiger-Müller counter to the undisturbed tissue.*

Metabolism and Salt Accumulation with Special Ref- erence to Barley Roots: — Returning now to the main discussion, following the earlier work on *Nitella* cells, F. C. STEWARD (1932) with the cooperation of several members of our laboratory, began a series of re- searches on potato tuber tissue. This relatively uni- form tissue was prepared in the form of thin discs and the processes of ion absorption and accumulation were followed under carefully controlled conditions in the culture solution in which the discs were immersed. The bromide ion was again found to be very useful

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*It is desired to acknowledge here the indispensable work of Dr. P. R. STOUT in the many studies with radioactive isotopes made in the laboratory of Plant Nutrition, and also the coopera- tion of Prof. E. O. LAWRENCE and members of the Radiation Laboratory.

as a test ion. It would require considerable time to describe this extensive investigation with its many ramifications which was pursued later in England. For my present purpose it may suffice to say that one general conclusion derived from the experiments was that the well aerated cells at or near the surface of the potato discs possessed a marked capacity to accumulate mobile ions, like potassium and bromide ions, and that this accumulation was dependent on cell metabolism reflected by, but not stoichiometrically related to, the aerobic production of CO_2. In later researches STEWARD has emphasized also the capacity of the potato tuber cells to grow and to synthesize protein as a condition for salt accumulation, that is, that type of salt accumulation which results in increase in total salt content (both cation and anion) content of the cell.

From the point of view of soil and plant interrelations paramount importance must of course be assigned to the absorption and accumulation of salt by root cells, and we have given much attention to this subject for some years (HOAGLAND and BROYER, 1936). In order to simplify the system to be studied many of the experiments have been done on excised roots of young plants, especially roots of young barley plants. An earlier investigator had reported experiments along this line, but he did not succeed in causing his excised roots to absorb salt actively and concluded that rapid salt absorption is dependent on the attachment of the root to the shoot. Our own first experiments were not very successful, but a simple technique was later developed for the production of excised roots that had a remarkably high capacity for accumulating certain ions, notably potassium, halide and nitrate ions, over a limited, but adequate period of time for the purpose of the experiments.

Within a relatively few hours the salt content of the roots could often increase by several hundred per cent. Thus we had admirable material for the study

of the influence of various factors on salt accumulation. The roots of barley plants approximately three weeks old that possessed this high capacity for salt accumulation were initially low in salt, but not starved to the point of injury or diminished root growth. They had a high initial sugar content. This status of the roots was the result of the regulation of the amounts
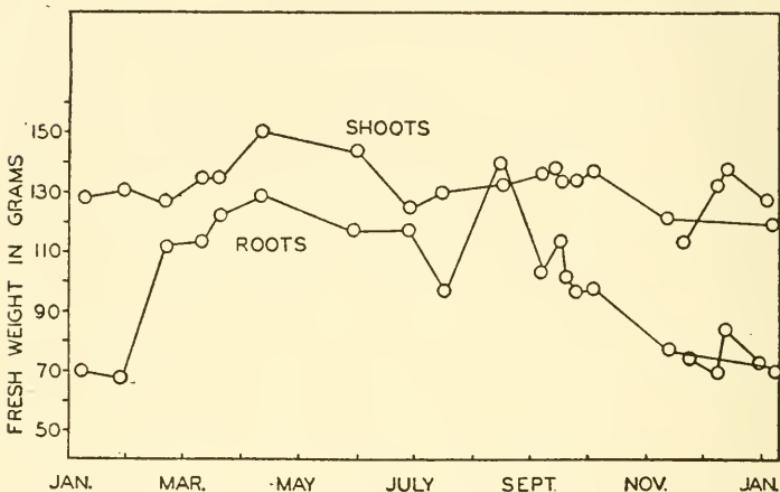
TEXTFIGURE 9. — Relative fresh weight of roots and shoots of young barley plants grown by the same technique for equal periods of time at different seasons of the year in a greenhouse (Berkeley, California). The composition and volume of nutrient solution were constant. There is reason to believe that the large changes in root-shoot ratios were largely dependent on the varying intensity and quality of the illumination. At certain periods of high illumination weights of the roots equal or exceed those of the shoots.

of nutrient salts made available during the preparatory growth period, and the selection of a suitable period of time for the latter. Not only entire root systems, but also single unbranched roots can be investigated by appropriate technique. (*See plates 17 and 18*).

It should be added that the most active salt absorbing roots were produced only during the seasons of good light. It is something of a digression from my main theme, but it is of interest to note that the

relation of root weight to shoot weight in the barley plants was notably influenced by variations in the natural illumination. The high proportion of root to shoot characteristic of summer grown plants could not be reproduced in the winter season by any manipulation of the culture medium. Extending the period of illumination with Mazda lights was also ineffective.
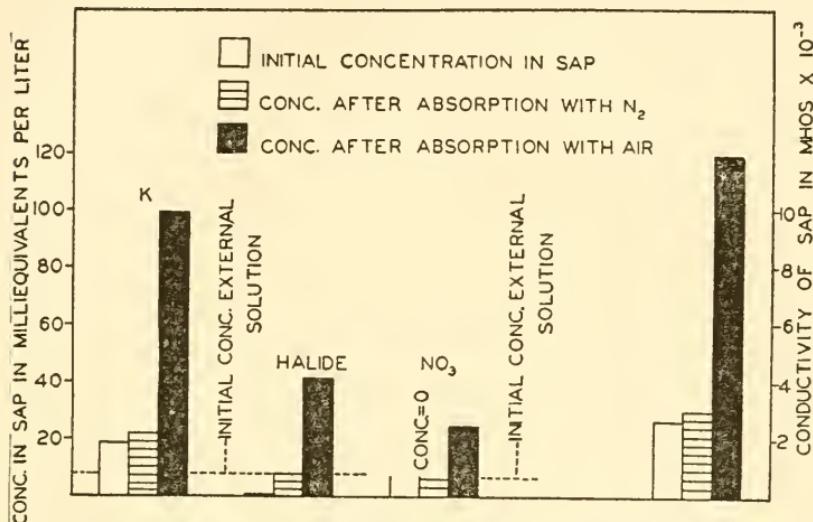
TEXTFIGURE 10. — Showing essentiality of aerobic metabolism for accumulation of potassium, halide and nitrate ions by excised roots of barley plants. By accumulation of ions is meant building up in the sap a concentration higher than that of the external solution. The conductivity of the sap rose to a much higher value than that of the solution under aerobic conditions, but not under anaerobic conditions.

One of the indispensable requirements for salt accumulation (movement of salt against a gradient) by excised barley roots is a supply of oxygen. The process is one definitely dependent on an aerobic metabolism, as is the accumulation of salt by potato discs. The stirring of the solution incident to aeration and the removal of CO_2 are also factors, but salt accumulation does not take place when CO_2 is removed by nitrogen gas bubbled through the solution. A fairly high

CO_2 concentration is required to depress greatly the process of salt accumulation. A very small oxygen tension in the gas stream will induce some salt accumulation to occur. In our recent experiments, however, in which all feasible precautions were taken to exclude oxygen, we have never observed any certainly significant movement of an ion into the plant against a gradient of concentration, as judged by analysis of expressed sap.
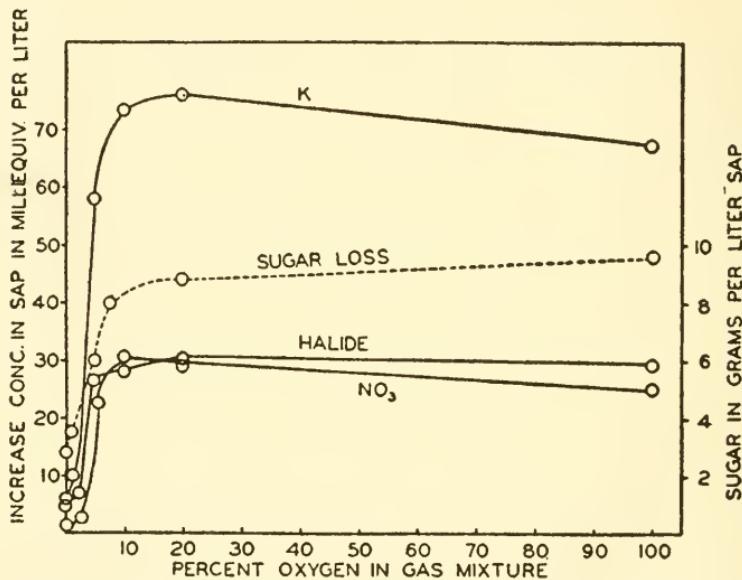
TEXTFIGURE 11. — Effects of oxygen in the gas stream supplied to excised barley roots on accumulation of various ions and on sugar utilization. (From HOAGLAND and BROYER, 1936).

Since metabolic activities dependent on or associated with aerobic respiration govern salt accumulation in the roots, it is to be expected that the temperature coefficient of the process should be high within certain biological temperature ranges. The expectation is borne out by the experimental evidence on this point. The temperature effect may not be revealed, of course, unless experiments are made on roots with a high salt absorbing capacity and in the

absence of other limiting factors. The temperature
acceleration of salt accumulation is not necessarily of
the same magnitude for all ions and thus the degree
of selective absorption of ions may be different at
different temperatures. With respect to the nitrate
ion consideration must also be given to the influence
of temperature on the reduction of nitrate in the root
tissues after nitrate ions have been absorbed.
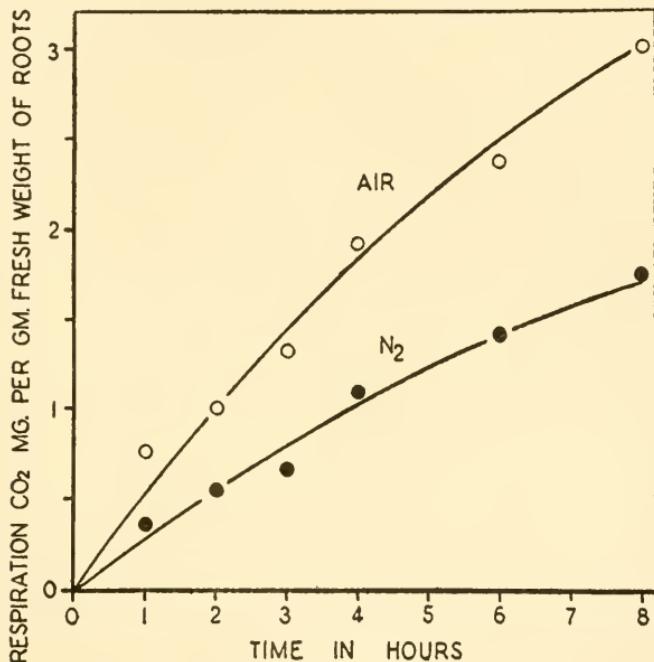
TEXTFIGURE 12. — Effects of bubbling air and N_2 gas
through the external solution on CO_2 production by ex-
cised barley roots. A large amount of CO_2 is evolved
anaerobically, but this does not result in salt accumula-
tion. (From HOAGLAND and BROYER, 1942).

Carbohydrate must be available for the respiratory
or other metabolic processes associated with salt ac-
cumulation. Direct evidence of this requirement was
furnished by experiments in which the sugar content
of the roots was reduced to a low value by aerating

the roots for some time following excision, while they were immersed in distilled water or in a solution of the relatively inert salt, $CaSO_4$. Then in a subsequent period it could be shown that marked increase in the capacity for accumulation of potassium salt resulted from the addition of sugar to the culture medium. Presumably certain growth substances are likewise required although direct proof of this is not easy to obtain under the conditions of these particular experiments. On the related processes of exudation to be
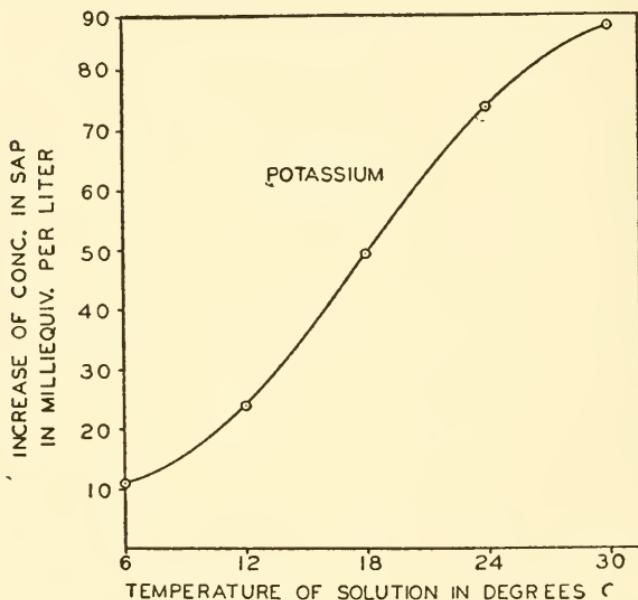
TEXTFIGURE 13. — Showing temperature curve for absorption of potassium by young excised barley roots. Other conditions were all favorable for salt accumulation.

discussed next time auxin effects were in evidence in the experiments of SKOOG, GROSSENBACHER and BROYER as carried on in Berkeley. It will also be recalled that in the culture of root tips, certain vitamin substances have to be supplied, especially vitamin B_1. In the present experiments it is reasonable to

infer that this vitamin was present in the roots because of previous translocation from the shoot.

The salt accumulation by the roots is notably selective, that is to say, some ions are absorbed more rapidly than others. Potassium is absorbed by the barley roots much more rapidly than calcium or magnesium. Sulphate is a very slowly absorbed ion, while nitrate and halide ions are absorbed with relative rapidity. These differential absorptions of ions imply ionic exchanges between the root and the culture medium and also changes in the hydrogen ion concentration of the culture medium. For example, from a solution of calcium nitrate, nitrate ions may be absorbed readily and accumulated in the sap as nitrate ions in higher concentration than that of the outside solution (some reduction of nitrate also takes place), while but little calcium enters the cells. An electrostatic balance must obviously be maintained in both the internal and external solutions and this is accomplished externally by bicarbonate ions taking the place of the absorbed nitrate ions in the culture solution, and internally by readjustments of organic acid content. From a potassium sulphate solution potassium ions are absorbed in excess of sulphate ions. As a consequence the acidity of the culture solution increases rapidly. In addition, calcium and magnesium ions enter the solution from the roots, possibly by secondary exchanges for hydrogen ions of the solution.

The readjustments of the metabolism of the roots, with a tendency to maintain hydrogen ion concentration in the root sap within relatively narrow limits are of considerable interest, but this is a matter for later consideration. Without citing the detailed evidence, I should stress now the point that in these differential absorptions, not only the physical-chemical properties of the ions are concerned but also the state of metabolism of the plant cells and their content of salt at any given time (HOAGLAND and BROYER, 1940). Further, it does not follow that two ions of a salt

will necessarily be absorbed at different rates. STEWARD has in fact emphasized that the discs of potato tuber usually absorb and accumulate potassium and bromide ions for a considerable period of time, in practically equivalent amounts. This equivalent absorption may also occur over certain experimental periods in studies on barley roots. This type of absorption has been called by STEWARD "primary" absorption as distinguished from those absorptions which involve ion exchanges, without net gain of salt by the cell, for which the term "induced" absorption has been proposed.

Finally, as part of this general survey, I should state that the absorption and accumulation of salt by a root is not uniform at all points. The apex is the most active region and the activity decreases at points successively farther from the apex, as PREVOT and STEWARD (1936) noted. Microrespirometer measurements in this laboratory and evidence by others indicate that oxygen consumption follows the same pattern.

Accumulation of Salt and Permeability: — I have considered some of the environmental factors and conditions of internal metabolism which lead to salt accumulation. Under what circumstances will the accumulated salt be retained in the cells? Interesting data bearing on this question have come from experiments in which radioactive isotopes have been utilized. Roots were allowed to accumulate a small amount of radioactive potassium and then, after rinsing, the roots were immersed in a large volume of distilled water (JENNY and OVERSTREET, 1938). Almost no potassium left the tissue as indicated by the extremely delicate test for radioactivity. According to this test the healthy roots were practically impermeable (as a net effect) to the outward movement of salt. Nevertheless, much more salt could have been caused to enter the cells under the influence of active cell metabolism.

This might seem to suggest a strictly one way transport or one way permeability. Before reaching this conclusion it is necessary to ask what happens when roots are placed not in distilled water but in a salt solution. With the aid of the radioactive isotopes the following kinds of observations have been made. Some radioactive potassium or bromide ions move out of the cell in exchange for non-radioactive ions of the solution. Yet at the same time the root tissue may show a net gain of salt (BROYER and OVERSTREET, 1940). As I have already pointed out, without the use of the tagged ions we should know only about the net change of salt content. Thus ions can leave the cells by some sort of an exchange process, but the rate of outward movement by ion exchange is slow in comparison with the rate of entry, even against a gradient, if cells have a high capacity for metabolic salt accumulation. The total exchange of potassium ions taking place over a considerable period of time comprises a relatively small percentage of the total potassium present in the roots. The possibility exists, as far as the data on the roots are concerned, that the exchange of potassium ions may be chiefly concerned with ions held by cell wall or protoplasm rather than the vacuole. This appears less probable for bromide ions. In the early experiments on *Nitella* cells marked chloride-bromide exchange was observed.

The active absorption and accumulation of salt against concentration or activity gradients has been stressed, but the question remains whether or not salt or its ions can enter the root cells when the gradient is *inward*, in the absence of aerobic metabolism. Seeking some definite information on this point, barley roots were subjected to solutions of potassium bromide of 50 to 60 milliequivalents per liter concentration (HOAGLAND and BROYER, 1942).

The roots were of low-salt type, so that the concentration of potassium was initially higher outside than in the sap. Despite this inward gradient and

the relatively high concentration of salt in the culture medium, little potassium or bromide entered the roots, relative to the external concentration, when purified nitrogen was bubbled through the solution. Concentrations in the sap did not rise to the outside level. The cells behaved as though their permeability to salt
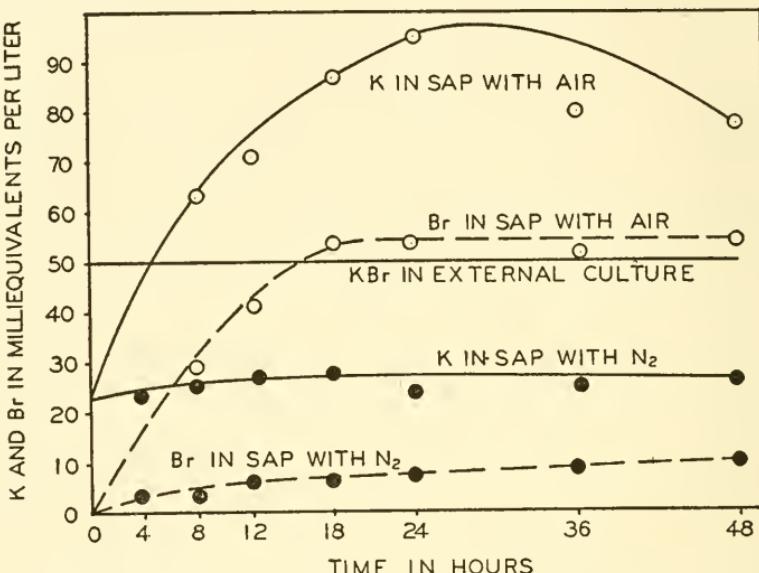
TEXTFIGURE 14. — Influence of aerobic conditions on salt accumulation by excised barley roots, from solutions of relatively high concentration. Initial concentration of potassium ions in external solution was higher than in sap of roots, but little potassium was absorbed in the absence of oxygen. With oxygen, concentration of potassium in sap rose to much higher level than in external solution. (From HOAGLAND and BROYER, 1942).

was very low. Under an aerobic condition the effect was notably different. Both potassium and bromide entered the cells rapidly. The concentration of potassium became much higher in the sap than in the external medium, even though the concentration of the latter was relatively large. Bromide ions accompanied the potassium ions in not far from equivalent quantity.

Before carrying the discussion farther I wish to draw your attention to the experiments of BLINKS
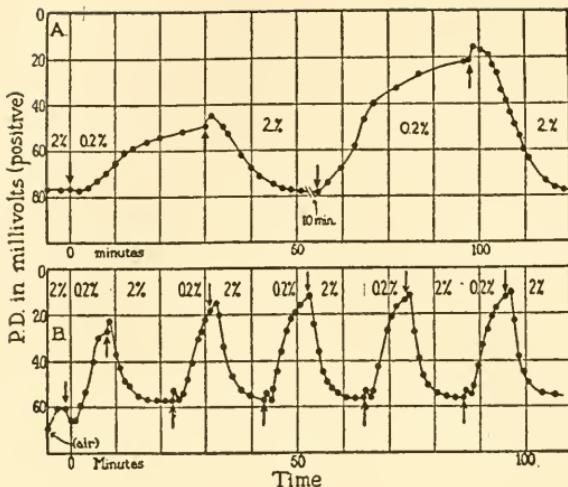
TEXTFIGURE 15. — Effects of oxygen on bioelectric potentials in *Halicystis*. Graphs showing fall of P.D. in impaled cells of *H. ovalis*, when 0.2 per cent O_2 in N_2 is substituted for 2 per cent O_2 (each bubbling continuously). In A the fall of P.D. in 0.2 per cent O_2 is rather slow the first time, possibly because air had not been thoroughly exhausted from the vacuole, although 2 per cent O_2 had been bubbled for $\frac{1}{2}$ hour previously (without reducing the P.D. below the air value). After recovery in 2 per cent O_2, the second fall in 0.2 per cent O_2 is faster, and reaches a lower level. Note the downward cusps preceding recovery in each admission of 2 per cent O_2.

In graph B another more sensitive cell is shown. This had 70 mv. P.D. in aerated sea water, which fell to 60 mv. in 2 per cent O_2. The fall on bubbling with 0.2 per cent O_2 is much faster and the P.D. drops to 18 mv. Readmission of 2 per cent O_2 promptly restores the P.D. The reproducibility of the curves and of the levels reached in each case are noteworthy.

Ordinates are P.D. in millivolts, the sign, outside of cell positive. Arrows indicate change of the gases bubbled. (From L. R. BLINKS, DARSIE, and SKOW, 1938).

and his colleagues (1938), on the large marine coenocytic alga *Halicystis*. An ingenious method had been developed for inserting capillary tubes in these cells in such a way that experimental solutions could be introduced into the interior of the cell, to replace the natural sap. Also bioelectric potentials across the protoplasm could be measured by a similar technique. So long as the cells remained healthy and had access to an adequate supply of oxygen, a steady bioelectric potential across the protoplasm was maintained. When deprived of oxygen the potential fell greatly, sometimes near to zero, but was restored when oxygen was again made available. This reversal could be repeated. Moreover, the direct current resistance of the cells was markedly increased under anaerobiosis. Numerous measurements of the effects of modifying the composition of the sea water medium on the bioelectric potentials, with and without oxygen supplied, were also carried out. The general interpretation of all these experiments was that differential ion mobility was immediately responsible for the bioelectric potentials, although their ultimate source had to be sought in metabolically released energy. The point of interest now is that the cells were considered to become more impermeable to salts in the absence of oxygen than they were in its presence, until the anaerobic condition resulted in irreversible changes in the cell, in the direction of injury.

COLLANDER (1939) from his experiments on large algal cells living in brackish water came to the conclusion that exchange of cations between the vacuolar sap and the outer medium was exceedingly slow — in other words, that protoplasmic membranes were highly impermeable to cations, at least. You will recall the experiments on *Nitella* cells I described earlier in this paper which showed that the cells were relatively impermeable with respect to the outward movement of salt to distilled water. A similar result based on experiments with roots was also noted.
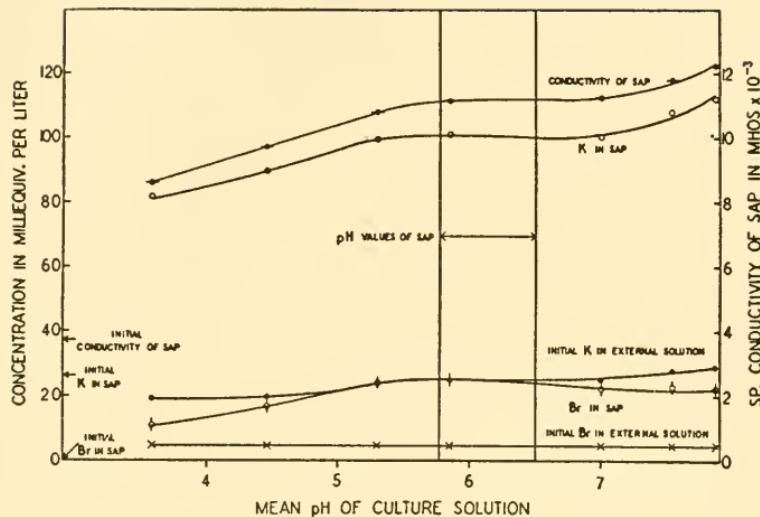
TEXTFIGURE 16. — An experiment with excised barley roots showing accumulation of salt (potassium and bromide ions) from buffered solutions at various *p*H values. At all *p*H values ions were found in sap at higher concentrations than those of the external solutions. Potassium could be accumulated when *p*H of the latter was either less or greater than that of expressed saps. Similar conclusions were supported by experiments in which root exudates were studied. (From HOAGLAND and BROYER, 1940).

Contemplation of the results of the investigations as a whole does not enable us to propose any general solution of the mechanism of salt accumulation by living cells; nevertheless we do perceive more clearly than before that ideas concerning permeability do not in themselves suffice. In fact, in metabolizing cells, permeability and active transport of salt are so inter-related that a separate treatment of the two concepts is exceedingly difficult. Tentatively, a general guiding view might be proposed that seems to be consistent with the data I have cited. This is that vacuolar membranes are relatively impermeable so long as the protoplasmic organization is not too greatly impaired. Salts can, nevertheless, be pumped into the vacuole with the use of metabolic energy, in the utilization of which an aerobic respiratory system is involved. The mechanism of the pump is not revealed, but there occurs apparently some preliminary combination of protoplasmic constituents with the solute and it is almost impossible to avoid some concept of ion ex-change as part of the general process of salt accumula-tion. As regards the application of energy to the salt accumulation process this remains an unsolved problem. All explanations become more difficult when we consider the upward movement of the salt absorbed by the roots. We shall find that ions can be accumu-lated by root cells and subsequently they can leave these cells to move upward and be accumulated by cells in the upper part of the plant. A polarized move-ment of salt is implied.

General Remarks: — My discussion so far has been rather narrowly limited to the accumulation of salt. It should not be assumed that living cells cannot also move organic substances inward or outward against gradients with the utilization of metabolic energy, for there is reason to believe that this does at times occur. Indeed the accumulation of salt by plant cells seems to be but one aspect of a general attribute of living

cells. HÖBER (1940) has reported evidence of secretory activity for certain kinds of dyes by kidney and liver cells. Several theories of sugar transport in animal or plant tissues invoke metabolic energy in one form or another.

Many attempts have been made to construct artificial cells to imitate, at least in some respects, the action of living cells in their absorption or accumulation of inorganic solutes. The general observation might first be offered that even if in an artificial system some function similar to that of the living cell is performed, there is no necessary conclusion that the cell achieves the same step in the same way. Actually it does not appear that any artificial cell so far proposed can accumulate salt in the same way as does the living cell.

One ingenious artificial cell has been devised in which a layer of guaicol or similar substance represents the protoplasm, and by maintaining a hydrogen ion gradient between inner and outer compartments of the cell — that is, the pH is higher outside than inside — potassium accumulates (OSTERHOUT, 1936). The theory is that KOH reacts with the guaicol or other substance and is released to the more acid solution inside. Undissociated molecules rather than ions are considered to be primarily concerned in the initial process of salt entry. The accumulation of anions is not, however, directly accomplished, and many results on living cells, including some reported on *Nitella*, and extensive studies on roots in our laboratory, do not appear to be consistent with the view that a pH gradient of the kind suggested is indispensable to the accumulation of potassium by plant cells.

I do not wish to argue that important facts may not be learned by the study of artificial cells or membranes. We may expect that modern research on oriented mono- and polymolecular films will have the most valuable consequences for biology. What I do wish to stress is that the solute movements in living

cells are so intimately related to and dependent on processes of metabolism that it should not be hoped that any real or hypothetical artificial cell or membrane from which the factors of complex metabolism are absent, can go far in imitating the processes by which living cells absorb and accumulate solutes. It is an unfortunate fact in this field of inquiry that living cells often operate in a manner inconsistent in one respect or another with mechanisms conceived to explain their operation.

REFERENCES:-

BLINKS, L. R. Protoplasmic potentials in *Halicystis*, IV. Vacuolar perfusion with artificial sap and sea-water. Jour. Gen. Physiol. 18: 409-420, 1935.
—— ——. The relations of bioelectric phenomena to ionic permeability and to metabolism in large plant cells. Cold Spring Harbor Symposia 8: 204-215, 1940.
—— ——, DARSIE Jr., M. L. and SKOW, R. K. Bioelectric potentials in *Halicystis*, VII. The effects of low oxygen tension. Jour. Gen. Physiol. 22: 255-279, 1938.
BROOKS, S. C. The intake of radioactive ions by living cells. Cold Spring Harbor Symposia 8: 171-180, 1940.
BROYER, T. C. and OVERSTREET, R. Cation exchange in plant roots in relation to metabolic factors. Amer. Jour. Bot. 27: 425-430, 1940.
COLLANDER, RUNAR. Permeabilitätsstudium an Characeen, III. Die Aufnahme und Abgabe von Kationen. Protoplasma 33: 215-257, 1939.
HOAGLAND, D. R. Salt accumulation by plant cells with special reference to metabolism and experiments on barley roots. Cold Spring Harbor Symposia 8: 182-194, 1940.
—— ——, and BROYER, T. C. Accumulation of salt and permeability in plant cells. Jour. Gen. Physiol. 25: 865-880, 1942.
—— ——, —— ——. General nature of the process of salt accumulation by roots with description of experimental methods. Plant Physiology 11: 471-507, 1936.
—— ——, —— ——. Hydrogen ion effects and the accumulation of salt by barley roots as influenced by metabolism. Amer. Jour. Bot. 27: 173-185, 1940.
—— —— and DAVIS, A. R. The intake and accumulation of electrolytes by plant cells. Protoplasma 6: 610-626, 1929.
HÖBER, RUDOLF. Correlation between the molecular configuration of organic compounds and their active transfer in living cells. Cold Spring Harbor Symposia 8: 40-50, 1940.

JENNY, H. and OVERSTREET, R. Contact effects between plant roots and soil colloids. Proc. Nat. Acad. Sci. 24: 384-392, 1938.

OSTERHOUT, W. J. V. The absorption of electrolytes in large plant cells. Botanical Review 2: 283-315, 1936.

PREVOT, P. and STEWARD, F. C. Salient features of the root system relative to the problem of salt absorption. Plant Physiology 11: 509-534, 1936.

STEWARD, F. C. The absorption and accumulation of solutes by living plant cells. I. Experimental conditions which determine salt absorption by storage tissues. Protoplasma 15: 29-58, 1932.

—— —— and PRESTON, G. Effects of pH and the components of bicarbonate and phosphate buffered solutions on the metabolism of potato discs and their ability to absorb ions. Plant Physiology 16: 481-519, 1941.

—— —— —— ——. The effect of salt concentration upon the metabolism of potato discs and the contrasted effect of potassium and calcium salts which have a common ion. Plant Physiology 16: 85-116, 1941.

—— ——, PREVOT P. and HARRISON, J. A. Absorption and accumulation of rubidium bromide by barley plants. Localization in the root of cation accumulation and of transfer to the shoot. Plant Physiology 17, 411-421, 1942.

VIETS Jr., FRANK G. Effects of Ca and other divalent ions on the accumulation of monovalent ions by barley root cells. Science 95: 486-487, 1942.

UPWARD MOVEMENT AND DISTRIBUTION OF INORGANIC SOLUTES IN THE PLANT

I have considered the question of the absorption and accumulation of inorganic solutes by excised roots, an approach that was found convenient as a way of describing, with the minimum of complicating circumstances, the general nature of the physiological processes of salt accumulation by plant cells. Obviously, in the growing plant, a large portion of the nutrient salt absorbed is translocated to the upper part of the plant, there to serve the functions of growth and metabolism in the various above-ground organs.

At one time this upward movement of salts was at least by implication regarded by many botanists as a simple matter. The role of transpiration in the upward translocation of inorganic solutes was frequently stressed. I can illustrate the point of view by a quotation from the text on botany of STRASBURGER:

> "Although a large amount of water is retained in the plant body for the maintenance of rigidity and enlargement of the organs, a still larger quantity of water taken up by the roots passes through the plant merely as a medium for the transport of nourishment. As the watery fluid absorbed by the roots contains salts, oxides and other non-volatile substances in solution, these on evaporation are left in the plant and gradually increase in quantity.
>
> "All those contrivances in plants, therefore, which render possible or promote evaporation operate chiefly in the service of nutrition."

Then a later development represented a swing of the pendulum in the opposite direction. The idea was sometimes advanced that movement of salt and of water had no definite relationship. In general the earlier teaching and the one that still prevails, was that the salts move upward in the dead conducting elements of the plant in the xylem tissues, along with water, but several investigators have not accepted this as the chief path of movement of salt and proposed instead that the main path of upward translocation may be in the living cells of the phloem. There also has long been discussion of the problems of secondary movements of solutes in living cells of the phloem and of the distribution of nutrient salts in various parts of the plant. This phase of the subject would require special treatment.

Metabolism and Salt Absorption and Movement — relations of water absorption to salt absorption: — I wish now to leave these broad questions in temporary suspense while I cite a number of simple experiments which may perhaps bring into sharper focus several aspects of the absorption and translocation of inorganic solutes. Concerning the general relations of water and solute absorption certain facts presented in the previous lecture should be recalled. They made clear that excised barley roots may have a high capacity for salt absorption and accumulation over limited intervals of time, during which the roots maintain a suitable metabolic activity by the utilization of organic substances previously stored. There are even instances of the absorption during a short time of nearly the same amount of salt by excised roots as by corresponding intact, transpiring plants. It is apparent, therefore, that the absorption of salt and the absorption and transpiration of water are in this respect independent processes. The continued absorption of salt is nevertheless dependent on the functions

of the green parts of the plant and it becomes of interest to observe, under several known conditions, the relation of water absorption to salt absorption by the transpiring plant.

The information sought cannot be obtained by some of the older methods employed in the study of the relation of water absorption to salt absorption or movement; for example, by comparison of the ash
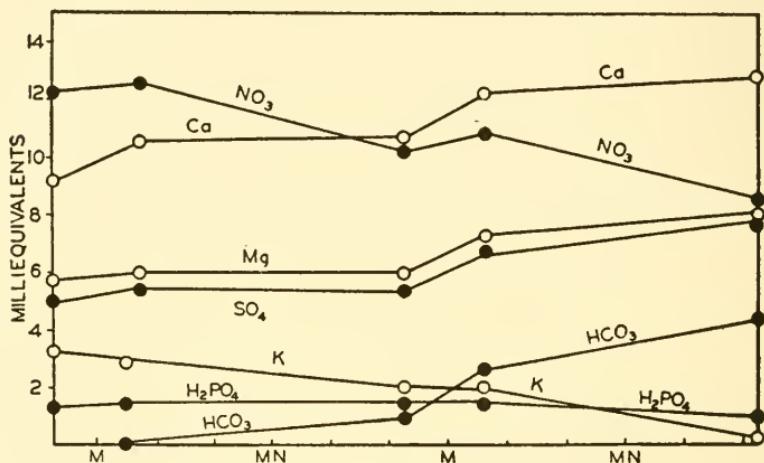
TEXTFIGURE 17. — An early experiment illustrating changes in ionic concentrations in a nutrient solution caused by absorption of water and nutrients by barley plants. Note changes in concentrations of individual ions and appearance of HCO_3 ions in appreciable concentration. Thus excess absorption of anions over cations is compensated. (From HOAGLAND, 1923, redrawn).

content of plants grown for a long period in shade and in full light. Entirely different types of plant systems are thus developed and the interpretation of data presents too great difficulty and complexity to afford answers to the questions we are now proposing. Simple experiments conducted over brief periods of time with initially uniform plants previously grown under appropriate control of nutrition are needed.

Let us first consider an experiment in which young metabolically active barley plants are absorbing water

and salt over a 24 hour interval, avoiding significant loss of water from the nutrient solution other than that which occurs through the plant itself. Under these circumstances, what changes will ensue in ionic concentrations and proportions in the nutrient solution as a net result of the absorption of both water and ions by the plant? The illustrative data available prove
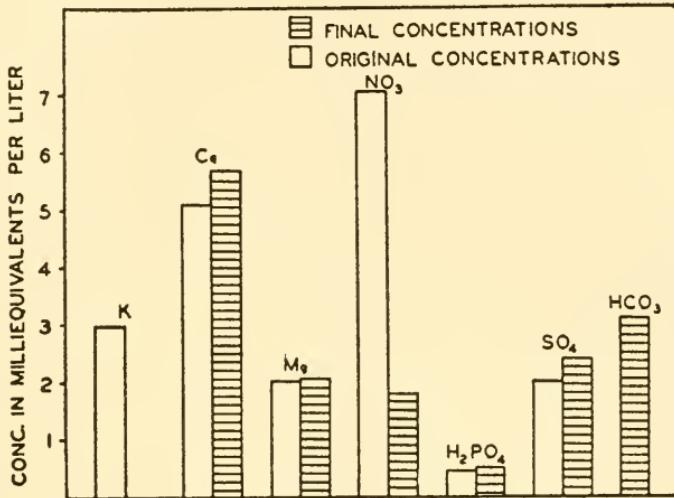
TEXTFIGURE 18. — Changes of concentrations in a nutrient solution as barley plants absorb water and ions. Relatively, a greater quantity of the ions than of water is absorbed in some cases, so that the solution is diluted. This is notably true of potassium ions in the above experiment of 24 hours duration. The concentration of potassium sank almost to zero. Some ions increase in concentration, because water is absorbed in relatively greater amount than ions.

that ions can be absorbed from a culture solution either more or less rapidly than water, depending on the nature of the ions, and on all the other factors governing the absorption of water and solutes. The importance of factors of metabolism is indicated by subjecting similar sets of plants to different root temperatures or to different oxygen supplies to the roots. The relation of salt absorbed to water absorbed varies

greatly, under the influence of these factors of root environment, even when the same nutrient solution and the same aerial environment are provided for the plants. Further, at any given period the amount of salt absorbed by the plant per unit of water absorbed

ABSORPTION AND TRANSLOCATION OF SALT IN A CUCURBIT

Conditions	Water Absorbed c.c.	Absorbed from solution Total m.e.		Br in sap Total m. e.			Total
		K	Br	Leaves	Stems	Roots	
Light - roots aerated	520	9.2	8.4	2.4	3.6	1.3	7.3
Dark - roots aerated	90	10.5	8.8	1.7	4.8	1.5	8.0

In another experiment a large plant rapidly trans-
piring, but with roots in a non-aerated solution,
absorbed in the roots very little Br end none was
translocated to upper parts of the plant. Large
amounts of Br were absorbed and translocated in plants
with aerated roots.

TABLE 3. — Absorption and translocation of salt in a cu-curbit as affected by conditions influencing transpiration and by aeration of culture solution. (From BROYER).

will depend on the amount of salt already absorbed and on available sugar and probably other organic substances stored in the roots.*

Another simple experiment will illustrate the view that salt does not normally move into a plant merely in relation to the water absorbed. A large and actively transpiring squash plant had its roots placed in a solution containing bromide, with insufficient aeration of the roots. A little bromide entered the roots, but none at all, within experimental error, was found in the stems or leaves. A similar plant which had its roots well aerated not only absorbed much more bromide but also translocated significant amounts to all parts of the shoot. I may add that squash roots have a high requirement for aeration under ordinary water culture conditions.

*Early experiments of this laboratory (HOAGLAND, 1923) gave definite evidence on the differential absorption of water and solutes but the full implications of the experiments were not appreciated at the time.

We can also make experiments bearing on the general problem under discussion by comparing the amounts of salt absorbed and translocated over a limited time period by young barley plants held in one case in the dark in a highly humid atmosphere, and in another, exposed to illumination and to relatively low humidity of the atmosphere, inducing high trans-
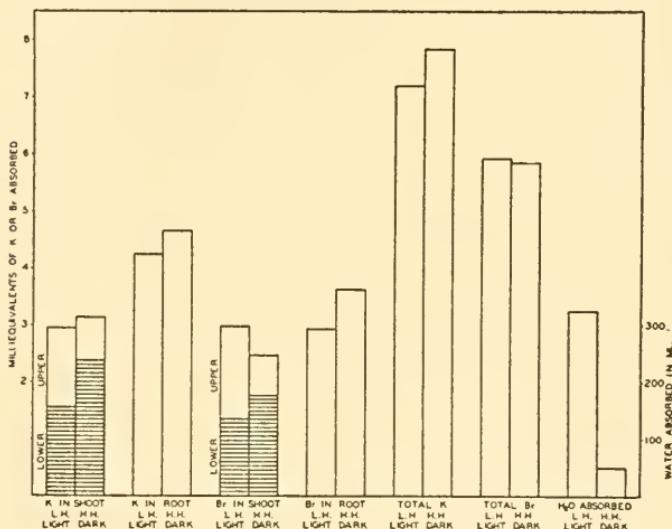
TEXTFIGURE 19. — Absorption of K and Br by young barley plants over a short experimental period (12 hours). Some plants were illuminated and in a relatively dry atmosphere; others were placed in darkness in a very humid atmosphere. Large differences in water absorption are shown, but there are no corresponding effects on salt absorption. (BROYER and HOAGLAND).

piration. When experiments of this kind are made with barley plants of low initial salt status and high in sugar content, the total salt absorption may be approximately the same under the two widely different environments. Further, the general distribution of salt between the root and the shoot may not be greatly affected by the aerial environment, although within the shoot itself the salt may be distributed differently

between the upper and lower parts as affected by conditions producing low or high transpiration, when these are in marked contrast.

It is helpful to describe still another experiment with young barley plants which was simple in design but involved a large amount of detailed work.* In this experiment plants were grown for several weeks
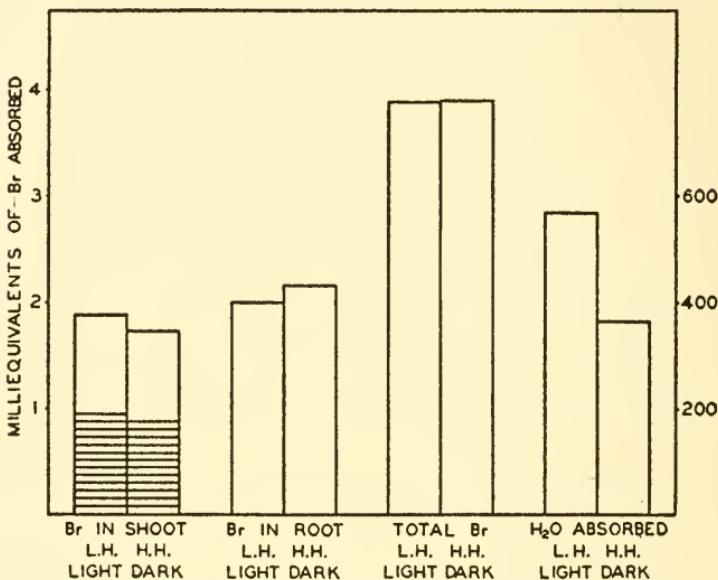
TEXTFIGURE 20. — Absorption and translocation of Br ions in young barley plants under two environmental conditions, in controlled chambers for maintaining desired temperature and humidity. (BROYER and HOAGLAND).

with three major differences in the manner of presentation of inorganic nutrients to the plants: (a) nutrients were made available the entire 24 hours of each day; (b) nutrients were available only 12 hours out of 24, with illumination of the plants most or all of the 12 hour period (depending on the time of year);

*This experiment was carried out by T. C. BROYER and K. A. GROSSENBACHER at the writer's suggestion.

(c) nutrients were available only during a 12 hour dark period in each 24 hours. During periods of exclusion of nutrients, the roots were immersed in distilled water.

The plants were grown in a greenhouse for three-week periods according to this plan, in both summer
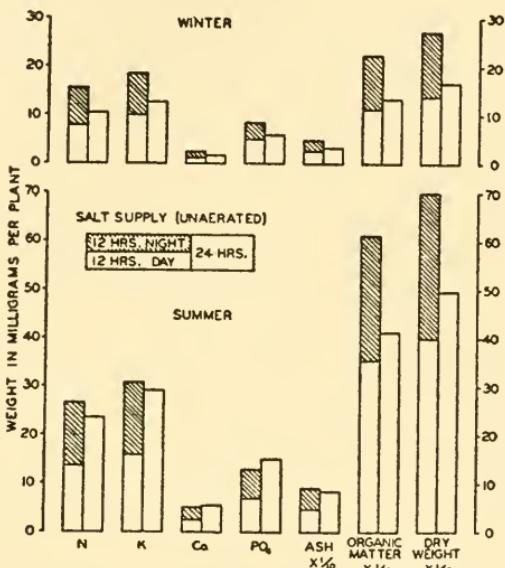
TEXTFIGURE 21. — Total amounts of nutrients under several conditions as explained in text. These plants called "unaerated" actually had access to considerable oxygen, but did not receive forced aeration. The general conclusions are similar for other sets receiving such forced aeration. (From BROYER and HOAGLAND).

and winter seasons. They made excellent growth with all three modes of supplying nutrient salts. The data of these experiments provided evidence that with respect to the ions studied (K, Ca, Mg, H_2PO_4, NO_3) their intake by the plant over a 12 hour interval was nearly the same during a dark period, with relatively small water absorption by the plants, as during a

period of illumination, with relatively large absorption
of water. In summer the total salt absorption for 24
hours was approximately double that for 12 hours,
but in winter, with its deficit of light energy restrict-
ing growth and metabolism, the total absorption for
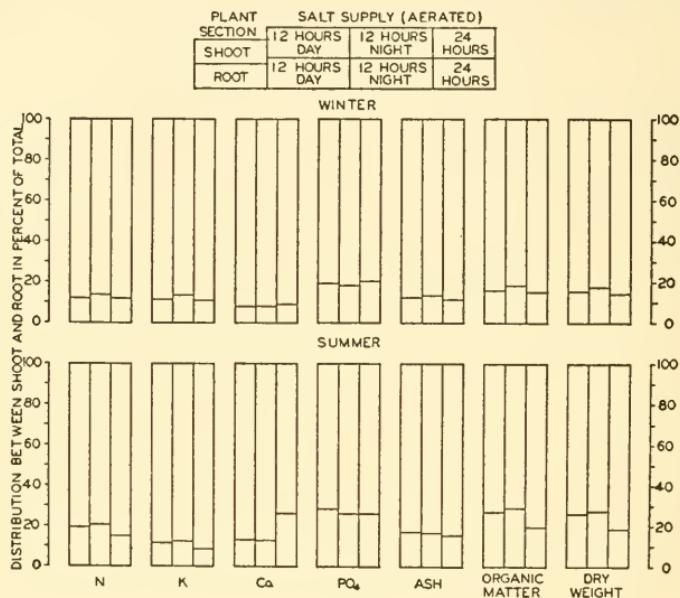
TEXTFIGURE 22. — Distribution of various nutrients in
barley plants between root and shoot under various meth-
ods of supplying salt to the plants, *i.e.* during day or
night or for full 24 hours. (Each set of 3 blocks repre-
sents the 3 conditions). Experiments are shown for sum-
mer and winter seasons. In all cases most of the nutrients
were found in the shoots. (Shown by vertical distribu-
tion). (From BROYER and HOAGLAND).

24 hours was considerably less than twice that for a
12 hour period.

Evidently the plants were at work absorbing salt
the full twenty-four hours, when salt was made avail-
able to them for this period. It was not the movement
of water that chiefly determined the amount of salt
absorbed, but rather the metabolic activities of the

plant, which were dependent upon the availability of energy containing compounds or special organic units, and of substances catalyzing or regulating metabolism, for all of which photosynthesis is ultimately essential. In these experiments not only the absorption of salt but likewise its distribution between the root and the shoot was largely independent of the quantity of water absorbed and given off by the plant. Essentially, therefore, the salt absorption and movement was dependent on the climatic factors that determined carbon fixation, respiration and other biochemical processes.

It is possible to arrange other experimental conditions which might seem to lead to a divergent conclusion but when the data are critically analyzed the general view just outlined is supported rather than contradicted. Barley plants were grown in a preliminary growth period under two contrasted nutritional environments. One set received frequent changes of nutrient solution which produced a high-salt and low-sugar status in the plants; the other set, as a result of limitation in the nutrients supplied during the preparatory growth period, attained a low-salt, high-sugar status. Both sets of plants were then subjected for a short time (about 24 hours) to several conditions of illumination and humidity and the absorption and upward movement of potassium and bromide ions were studied. The roots received full aeration in all cases.

The importance of the initial status of the plant on subsequent salt absorption and movement during an experimental period was manifest. The influence of aerial environmental conditions on the intake of salt was much greater for the initially high-salt, low-sugar plants than for the low-salt, high-sugar plants. The former responded far more in increased salt absorption to conditions inducing increased transpiration, but these conditions also modified the metabolic status of the plants. Illuminated plants synthesized

TABLE 4:—

Influence of transpiration on absorption of salt by barley plants of low-salt, high-sugar; or high-salt, low-sugar status.

Experimental conditions	Water absorbed in ml. per gram fresh wt. shoot	Salt absorbed in milliequivalents $\times 10^2$ per gram total fresh wt. (loss from culture)		Total sugar (final)in expressed sap in gm. per liter		Computed bromide concentration in translocatory fluid to shoot in meq. per L.
		K	Br	Shoot	Root	
High-salt plants; low humidity, light	8.10	5.20	6.07	5.4	1.1	6.7
High-salt plants; high humidity, light	2.58	3.24	4.24	2.9	0.7	14.8
High-salt plant; high humidity, dark	1.49	1.59	2.15	0.3	Trace	9.8
Low-salt plants; low humidity, light	9.60	10.85	9.52	15.3	3.5	9.7
Low-salt plants; high humidity, light	3.60	10.40	9.65	6.2	2.5	25.1
Low-salt plants; high humidity, dark	2.52	8.75	9.13	2.0	0.8	27.8

The initial sugar concentrations were not determined, but must have been much higher than at end of experiment, especially for the high-sugar plants.

sugar. This was less important for the initially high-sugar plants than for the initially low-sugar plants. Furthermore, the largest increment of growth during the experimental period was made by the initially low-sugar plants exposed to illumination and low humidity.

Experiments on Exudation and Root Pressure: — I shall now turn to a special phase of salt movement that has some relation to certain of the experiments I have already described. I refer to the phenomena of which root pressure is a manifestation. Something of the general nature of this physiological response has been known for a long time, but its causes and significance are still frequently the subject of debate. In many young plants—barley or cucurbit plants are good examples—conditions of root pressure are made evident when transpiration is suppressed, by bleeding from cut stems, or by guttation, the extrusion of drops of liquid from the tips or margins of leaves. The study of the bleeding sap that originates in the conducting vessels of the xylem is of considerable interest for our present inquiry. This sap, as several investigators have found, can attain a relatively high concentration of inorganic solutes, higher than their concentration in the external nutrient solution. This may also be true of the guttation fluid, which is often far from being pure water, as is sometimes assumed.

More than one cause of root pressure can, perhaps, be effective but in young barley plants a definite interrelation was found in our experiments* between the absorption and movement of salt and bleeding or guttation. If the roots are immersed in distilled water, even though ample aeration is provided, guttation will soon cease or become slight in rate. If the roots are placed in a dilute salt solution without

*T. C. Broyer and D. R. Hoagland, unpublished.

aeration, guttation becomes negligible. If, on the other hand, the roots are immersed in a dilute salt solution containing mobile ions, are well-aerated, and kept at a favorable temperature, rapid guttation continues for a long time in a humid atmosphere. Very little guttation was observed when roots were exposed to a low temperature (5° C.). In brief, the development of root pressure seems to follow or to accompany an active transport of salt into the xylem conducting system, where the osmotic value rises above that of the external solution. According to one theory, water withdrawn from the external solution will depend on the difference between the osmotic pressure of the latter and that of the xylem vessels after intervening cells have reached full turgor.

An earlier view of PRIESTLEY that the osmotic gradient is brought about through release of organic solutes from differentiating cells does not seem to be applicable here. The response to conditions affecting salt movement is too rapid and further, the composition of the exuded fluid is chiefly inorganic.

The observation on guttation and on bleeding may argue for a simple osmotic movement of water, with the cell activities confined to the antecedent or concomitant movement of solutes. Yet this is not certainly the whole explanation. Some investigators believe that an active transport of water occurs under the influence of metabolic activities of the cell, possibly associated with the movement of salt, as for example in some type of electro-osmosis. It does not appear that there has yet been presented any really conclusive proof of the active transport of water in such systems, but the question is not closed.

In any event, a simple explanation of the phenomena in their entirety in terms of osmotic relations does not seem to be adequate. The work of GROSSEN-BACHER (1938) in our laboratory several years ago on root pressure and rates of bleeding is indicative of

the complexity of causes and effects involved in these processes. In the studies referred to observations were made of root pressures in decapitated sunflower or other plants kept under a nearly constant environment: that is, in dark chambers with controlled temperature and humidity. Despite this constancy of external environment, the plants passed through regular 24 hour cycles of variation in root pressure [compare also WHITE's (reviewed 1942) studies on single roots grown from root tips and earlier work by BARENETSKY (1877)]. The peaks of pressures—or
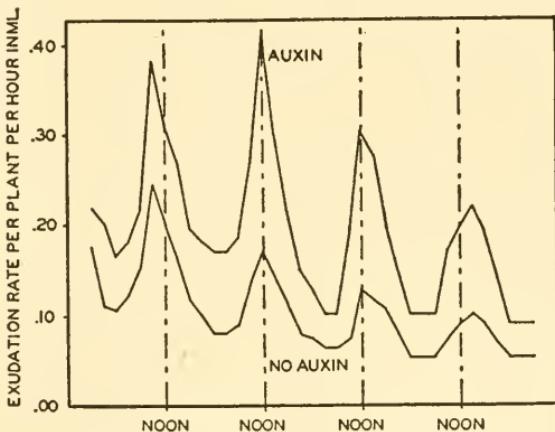
TEXTFIGURE 23. — Periodicity in exudation and effects of auxin application to *Helianthus* on the rate of exudation from decapitated plants at different periods in 24 hour cycles. (From SKOOG, BROYER and GROSSENBACHER, 1938, redrawn).

rates of exudation—were attained at periods usually not far removed from noon and the low points in periods around midnight. A protoplasmic rhythm is suggested, which governs the cell activities affecting salt and water movement into or out of the conducting system. By growing plants in continuous artificial light and cutting the stems at appropriate times, maxima and minima of pressure could be inverted, but the 24 hour cycle persisted.

The continuation of this research in association with Skoog and Broyer (1938) also produced evidence that an auxin plant hormone, beta indolacetic acid, could increase the magnitudes of the root pressures or bleeding rates, superimposed on the cycle I have just described, presumably through influencing the metabolic activities of cells that are interrelated with active accumulation or transport of salt.*

Another mode of approach to the problem (Hoagland and Broyer, 1942) of the secretory-like activity of root cells, which also furnishes certain further suggestions concerning the question of cell permeability, is found in the method of applying suction to the cut stem of a tomato plant and thus causing liquid to move through the root system under prescribed conditions in the culture solution environment. This is similar to the method of Kramer employed for studying water movement. Bromide was again chosen for convenience as an indicator ion. The plants were first subjected to an aerobic condition in the culture solution and liquid was collected from the cut stems. Bromide concentration rose to higher values in the recovered fluid than in the external solution. Then nitrogen was passed through the solution in one treatment, carbon dioxide in another and in a third treatment the passage of air through the solution was continued. With either the CO_2 or N_2 treatments there first occurred a sudden decrease in the concentration of bromide present in the liquid recovered from the plants. The concentration became lower than that of the external solution, whereas under the aerobic condition the concentrations remained higher. Soon, however, the curve for the CO_2 treated plant assumed an upward trend and the

*The writer is informed that two of the same investigators met difficulty in repeating these auxin experiments at Harvard University. A possible explanation of the discrepancy might be that different climatic conditions caused the production of plants with different potentiality of response to auxin.

concentration of bromide reached that of the outside solution. The curve for the nitrogen treated plant gave evidence only of a slight upward trend in the time allowed. Volumes of liquid moved through the roots under suction, per unit time, also were affected in a somewhat similar manner.
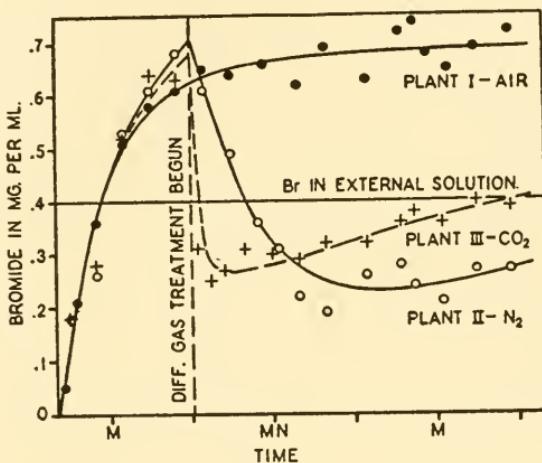
TEXTFIGURE 24. — Movement of Br into internal solution obtained by suction applied to stem of tomato plant. Roots of plants under several conditions: I, aerated, II, free N_2 gas bubbled through solution, III, CO_2 gas bubbled through solution. (From HOAGLAND and BROYER, 1942).

One way to interpret these results is to assume that the absence of oxygen, or the high concentration of carbon dioxide, caused loss of power of salt accumulation and at first a decrease of cell permeability, followed, however, by a breakdown of protoplasm and subsequent increase of permeability, but *without* any capacity on the part of the cells to secrete salt against a concentration gradient. The injurious effect of the carbon dioxide treatment is particularly evident and gives rise to a condition in which the unconcentrated salt solution is more or less readily drawn through the root system. With regard to the volume

of water passed through a plant, as KRAMER points out, this is much greater in a transpiring plant or when liquid is caused to pass through the system by applying suction than when simple exudation is taking place.
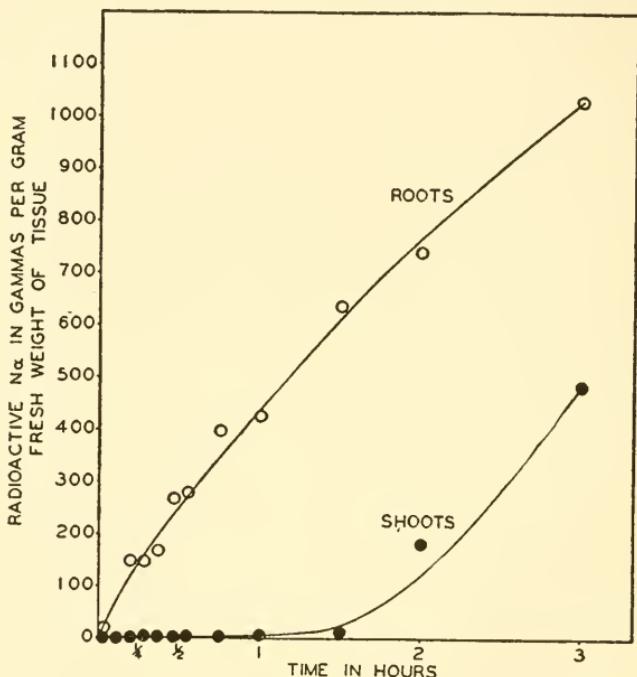
TEXTFIGURE 25. — Time curves for absorption of radioactive sodium and location in shoots and roots of young barley plants. Movement to shoot was delayed in initial period while roots were absorbing sodium, but later sodium moved to the shoot while roots were also still actively absorbing sodium.

All these experiments of various kinds suggest that salt can move through the root cells or out of them and into the xylem conducting system in the root by a process akin to secretion, at least as an overall effect, based on comparison of the external solution with the exudate. Several questions arise

with regard to this general concept. One of these is: Must salt first be accumulated by root cells to the limit of their capacity for holding salt, before upward movement begins? That this need not be so is shown by an experiment conveniently performed with the aid of radioactive tracers. For a very short period (one or two hours) practically all the absorbed salt

THE GRADATION OF CATION ACCUMULATION IN
ROOTS SHOWN SPECTROGRAPHICALLY.
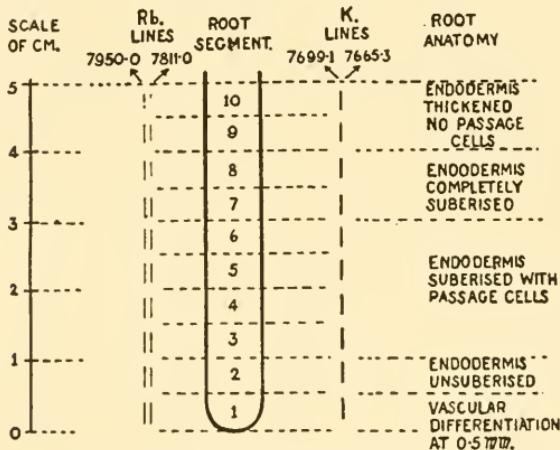
TEXTFIGURE 26. — Accumulation of salt in different regions of root. (From STEWARD, PREVOT, and HARRISON, 1942).

(radioactive sodium) was retained by the roots, but following this brief initial phase, the content of radioactive ions continued to increase in the roots and simultaneously upward movement to the shoot occurred, while the root cells were still far below the point of salt saturation.

Another question we might ask, thinking again of the intact plant, is: Assume that the plant is capable of further growth and that the root system does not have access to new supplies of a given ion

in the nutrient solution, to what extent can the shoot deplete the root of its previously accumulated salt? With bromide as the test ion, it has been shown that the growing shoot can gradually withdraw nearly all the bromide previously stored in the root cells. This was demonstrated in independent experiments by STEWARD, PREVOT and HARRISON (1942) and by BROYER and myself. Potassium can be reduced to a very low level of concentration, although naturally not all the potassium can be withdrawn from the root system. In a sense a competition for solutes occurs between the root and the shoot.

Why ions that have been accumulated by root cells and which can not be removed from the healthy cells by leaching with distilled water should nevertheless move out of these cells in the intact plant and upward to the shoot, is far from receiving a satisfactory explanation. The whole process involves a polarized movement of salt. PREVOT and STEWARD have remarked in an earlier paper:

> "The elucidation of those factors which in the *intact plant* cause this ready removal of electrolytes from root cells which have already accumulated them, and which in *excised roots* would necessitate drastic treatment with perhaps even irreversible changes, represents one of the most difficult and certainly one of the most fundamental of the outstanding problems."

As a sidelight on this problem the recent data of STEWARD, PREVOT and HARRISON (1942) on longitudinal distribution of potassium, rubidium and bromide ions in barley roots are worth citation. The spectroscopic technique was used in determining rubidium and potassium. In the excised root, the accumulation of these ions is most rapid at the apex and decreases at points farther removed from the apex. When the root is attached to the growing shoot the gradient tends to become reversed. STEWARD and his collaborators think that this may mean that the most active secretion of salt into the stele takes

place near the region of greatest metabolic activity at the apex, which in the excised root is also the region of most active salt accumulation.
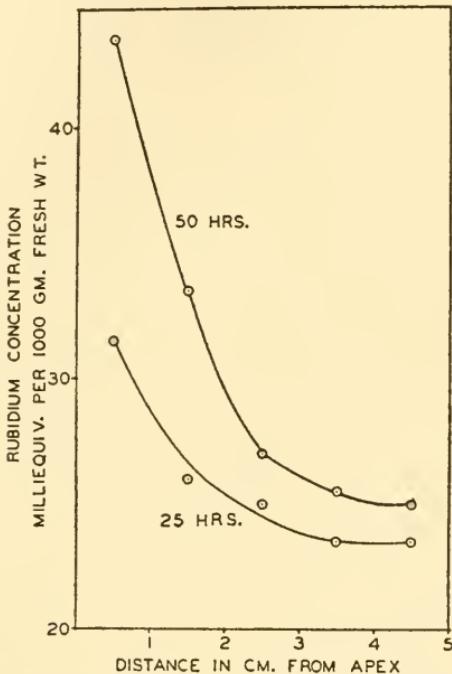
TEXTFIGURE 27. — Distribution of absorbed rubidium in excised barley roots with relation to distance from root apex, at two different time periods. (From STEWARD, PREVOT, and HARRISON, 1942, redrawn).

Another view advanced by CRAFTS and BROYER (1938), although based, as far as anatomical factors are concerned, on an earlier concept of PRIESTLEY, is that salt may be carried by protoplasmic connections from the well aerated cortex of the root into the less aerated central cylinder, where decreased metabolic activity causes loss of salt to the vessels of the xylem system. Even so, the net result would represent

from one point of view a process equivalent to secretion. According to this proposal it would not be essential that all the salt absorbed and transported to the xylem vessels first be accumulated in cell vacuoles. It is hoped to gain further information on this point by studies with isotopes.

Path of Upward Movement of Salt: — It becomes now desirable to consider further the question of inorganic solute movement and its accumulation in the upper parts of the plant before embarking on additional discussion of the interrelations of root and shoot.

As I have already said, several investigators have suggested the possibility that the upward movement of salt occurs primarily in living cells of the phloem, especially in the sieve tube system. Much of the original basis for this idea was found in experiments conducted over long time periods with woody plants, by the method of removing rings of bark from the branches to be subjected to examination. A difficulty with experiments of this kind is that secondary effects of ringing can occur, which may, for example, impair the nutrition of the roots, or lead to injury of the conducting system of the xylem. Transpiration may also be affected by accumulation of carbohydrate above a girdle. Despite these complications, CLEMENTS and ENGARD (1938) found in tests on one woody species, with a diffuse porous wood, that practically as much salt moved upward through a girdled branch as through an ungirdled one and that large amounts of salt moved upward past a girdle in all species studied although in most of them girdling decreased markedly the upward movement of salt. CLEMENTS, and also F. C. and A. G. STEWARD,* from another point of view, have emphasized the problem presented in

*Private communications.

woody plants by the need for salt to pass from old wood into new tissue.

Our own data on girdled cotton plants and on young citrus trees show that salt for a time may pass upward past a girdle almost as well as in a normal plant. The researches of MASON and his colleagues (1937-1940) will be recalled at this point.
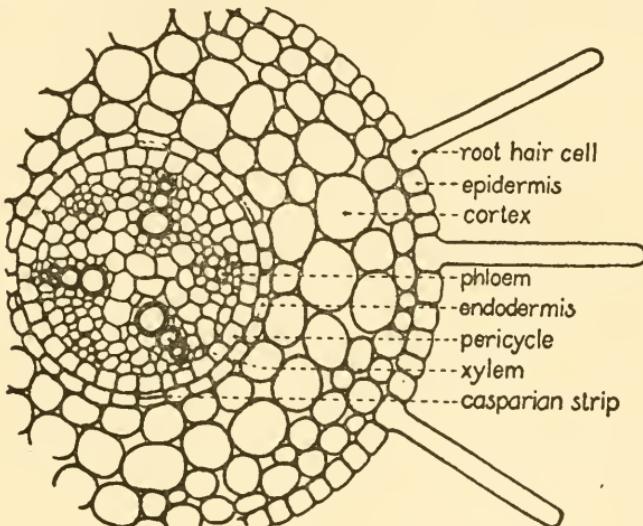
TEXTFIGURE 28. — Illustrating varying conditions of aeration in different anatomical regions. According to one hypothesis this would have a role in movement of salt to xylem system. (From CRAFTS and BROYER, 1938).

In some of our experiments with cucurbit plants a section of the stem was killed by steam and still salt could pass upward through the dead tissue for a short time with little impairment in the amount of salt moved. That organic nitrogen can move upward fairly readily in living tissue seems possible, but nitrate appears to move chiefly, if not entirely, in the wood.

The radioactive isotopes again furnish a convenient tool for the further study of the path of movement of

salt. The sensitivity of the method makes feasible tracing the movement of an inorganic solute even when the time interval is very short. Thus secondary effects occurring over longer periods are largely precluded. STOUT and HOAGLAND (1939) performed a series of experiments with radioactive tracers of phosphate, bromide, and potassium ions on several species of plants with the hope of gaining some rather clear cut data on the path of upward translocation of these ions.

The conclusion was that the upward movement of the solutes in the wood was far more rapid than in the bark, but that a rapid lateral transfer of solute from wood to bark took place whenever the two tissues were in contact. Thus the separation and analysis of the tissues sometime after absorption of the solute could not yield correct conclusions regarding the path of upward movement. As the salt moves upward it is rapidly accumulated on the way by metabolically active living cells. Extensive experiments in California by BENNETT* on the movement of radioactive potassium in prune trees girdled in various ways lead to similar general conclusions.

Other Effects Associated with Conditions Influencing Transpiration: — Another set of experiments in the laboratory was designed to test the effects produced by a highly humid atmosphere on absorption and upward movement of indicator elements in plants different in type from the barley plants already described. The purpose was to eliminate or to reduce transpiration to the lowest possible value by placing the plants in illuminated chambers with an atmosphere saturated with water vapor maintained by spraying water from fine nozzles.

In one experiment large squash plants were used in which the root pressure developed might not suffice

*In course of publication.
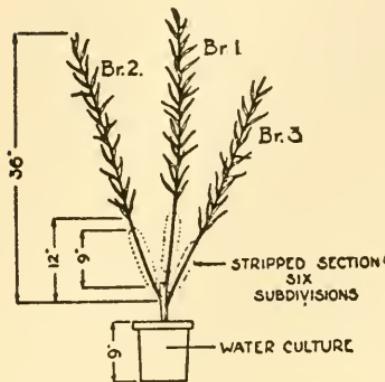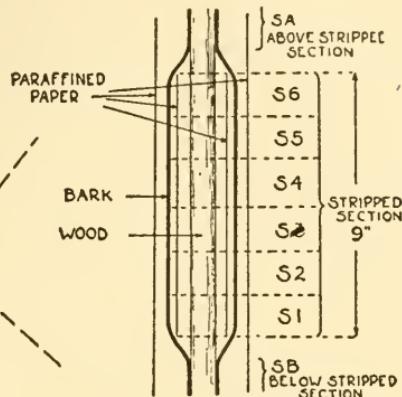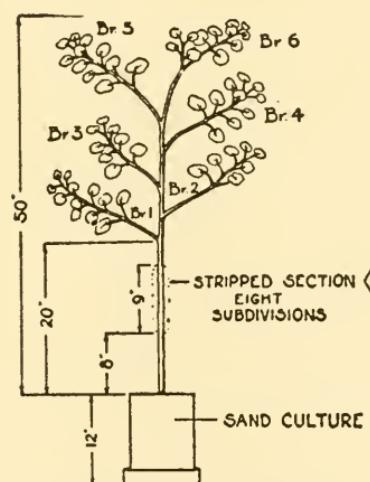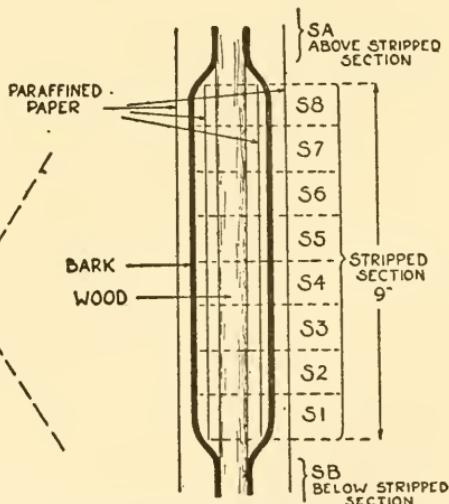
TEXTFIGURE 29. — Fig. 1, 1a, 2, 2a. Illustrating the method of stripping bark and of sectioning bark and wood in experiments with willow and geranium plants made for the purpose of measuring movement of radioactive elements. (From STOUT and HOAGLAND, 1939).

for accelerated movement of salt to the upper part of the plant. Radioactive bromide served as a test element. The suppression of movement of bromide to the upper part of the cucurbit plant was complete for a period of an hour or more. Other experiments on cotton plants with non-radioactive bromide gave similar general results. In the sunflower plant the distribution of bromide between the upper and lower parts of the shoot was definitely influenced by the humidity of the atmosphere, as far as the leaves were concerned. In this instance not much difference was noted in the bromide content of the stem under the two humidity conditions.

But the question cannot rest here. A plant in a humid atmosphere, even in the light, may be affected in other ways than by changes in the rate of loss of water as has already been indicated by one of the experiments with barley plants. Also, the picture would be incomplete without a reference to the relative accumulation of inorganic solutes by leaves, stems, and other aerial organs. Here, as in the roots, active uptake of solutes by living cells, under the influence of metabolism, assumes great importance. Various factors determine the capacity of the foliar cells to accumulate and to retain solutes. They are essentially the same factors that must be taken into account in explaining salt accumulation by roots. (Compare earlier general statement by STEWARD, 1935). Not only respiratory activity and associated processes, including growth, are involved, but also the salt status of the tissue at a given time; that is, the amount of salt already accumulated in relation to total capacity for accumulation. In experiments of this type low salt tissues of the shoot may be higher in sugar than high salt tissues, as was observed in the experiments on excised roots. In using the term salt in this connection the supply of nitrogen may be of special importance.

An experiment in this laboratory with squash plants illustrated the relation between the initial internal status of the plant and the external environment during the experimental period. Measurements were made by STEWARD of growth increments in leaf areas occurring during the absorption period. The largest accumulation of salt took place in those leaves of the high salt plants that increased most in area. These were the leaves on the plants in the light. In the low salt plants marked additional accumulation of salt occurred in the leaves in the dark, which, however, increased in area even under these conditions unfavorable for growth. The suggestion appears that the leaves, like the roots, of plants low in salt and high in sugar, maintain for a limited period a relatively high salt accumulating capacity, which is not immediately dependent on photosynthetic processes.*

Still another aspect of salt movement with a definite bearing on some of the questions raised above has been usefully studied by means of radioactive isotopes. This is the problem of the movement of ions to developing fruit of the tomato plant (ARNON, STOUT and SIPOS, 1940). Large plants were grown by water culture technique and the experiment was begun at a time when the plants bore many fruits at different stages of growth, from small green fruits to large ripe ones. Radioactive phosphate was then introduced into the culture solution and translocation of this newly added phosphate followed by means of a Geiger-Müller counter and by radio-autographs. After an initial period, the greatest accumulation of the newly

*Extensive experiments have been conducted in England by F. C. and A. G. STEWARD on cucurbits and on certain woody species, with reference to the factors of metabolism involved in the movement of inorganic solutes into leaves and growing points, with special consideration also of anatomical factors. The reports have not yet been published because of war conditions and while the writer is privately informed of these researches, it would not be appropriate to anticipate their future discussion.
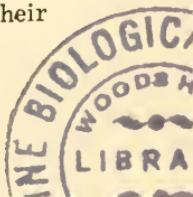
introduced phosphate occurred not in the regions of highest transpiration, but in the young still growing fruit. Comparatively little radioactive phosphate accumulated in the fully ripe fruit. Within the fruit itself the distribution was differential, and in general in accordance with the concept of potentiality for rapid accumulation of solutes possessed by growing cells with a high rate of metabolism. The highest concentration of phosphate was found in developing seeds. (*See plate 19*).

MASON and PHILLIS (1937, 1940) have proposed that something like a circulation of mobile inorganic nutrients can take place in the plant by which the solutes are carried in the wood to foliar regions, from which they may be reexported through living cells, even back to the roots. The supposition is that along the path of reexport some leakage into the xylem vessels may occur and the solutes be carried again upward. It is, of course, possible for previously accumulated solutes to move out of leaf cells to other regions of the plant. Experiments with radioactive elements give direct evidence of this—some backward movement as far as the roots may occur. Nevertheless, a circulation theory by itself seems too simplified and does not emphasize sufficiently the role of active processes in accumulation of solutes in both root and shoot.

General Discussion: — The question of secondary movements of salt in the living cells of the phloem is part of the general problem of translocation in this tissue. The theories evolved to explain this type of translocation are various and still highly controversial. Their detailed consideration falls outside the scope of my discussion. We need note now only that all theories at some point invoke the activities of living cells in the movement of solutes against gradients or in accelerating the processes of diffusion. The latter

idea has been developed and the term "activated diffusion", applied to movement of solutes in the phloem system. No direct experimental evidence is available for this hypothesis, which meets some theoretical difficulties. Indeed, one might well call attention to the fact that there is no approach to general agreement

TABLE 2. *Gain of phosphorus in sections of geranium after absorption period of 6 hours.*
(Calculated as PO_4 from radioactivity.)

		Gammas* PO_4 present in sections				P.p.m. (fresh wt.) PO_4 in sections			
		Bark	Wood	Petioles	Leaves	Bark	Wood	Petioles	Leaves
Branch	6	197	470	191	532	12	26	44	33
Branch	5	202	360	200	790	14	24	57	62
Branch	4	151	290	55	280	16	27	37	37
Branch	3	41	62	26	94	13	17	50	49
Branch	2	175	326	86	354	18	33	48	52
Branch	1	128	286	57	117	15	32	16	19
Stem above strip...	SA	270	860	..	..	16	37	..	..
	S8	9.0	112	..	..	4.5	44	..	..
	S7	.5	120	..	..	.28	44	..	..
	S6	.6	132	..	..	.3	49	..	..
Stripped section ...	S5	.8	138	..	..	.4	51	..	..
	S4	<.3	147	..	..	<.16	54	..	..
	S3	<.5	137	..	..	<.25	56	..	..
	S2	<.3	152	..	..	<.16	58	..	..
	S1	11.1	131	..	..	6.0	41	..	..
Stem below strip...	SB	316	442	..	..	24	41	..	..
Total gammas PO_4 moved past S2 = 7600									

* 1 gamma is equal to .001 milligram.

TEXTFIGURE 30. — Phosphate transport in garden geranium. (*See also* figure 29). (From STOUT and HOAGLAND, 1939).

by investigators on any mechanistic explanation of movement of solutes in the phloem.

I have now presented the results of various types of experiments bearing on the question of upward movement of inorganic solutes. It would be gratifying if these and other experiments could lead to a completed picture of these aspects of movement of solutes in plants. Unfortunately, our knowledge has

not advanced that far. Yet it may be permissible to outline in summary form certain general concepts that may usefully guide future research. First of all, the plant needs to be envisaged as an integrated organism with respect to solute absorption and movement as well as in other respects. The growth of the roots and the active absorption of salts from the external medium require a source of certain metabolites, which must normally originate in the shoot. Root metabolism is concerned in active transport mechanisms by which solutes are absorbed and carried into the conducting system. The concentration of solutes in the conducting system as the salt moves upward depends on the active transport capacity of living cells as well as on water movement.

The distribution of mobile salt ions in the various parts of the plant and their relative concentration in the different tissues is not merely a necessary consequence of the movement of water containing solutes. Here, too, relative rates of growth and metabolism of tissues determine the utilization or storage of the solute. Thus, transpiration does not assume the determinative role in either absorption or translocation of salt that was once supposed. Neither, however, can we dissociate entirely the absorption and upward movement of solute and of water. Transpiration makes possible the rapid carrying of nutrient salts over long distances and the rapid removal of salt from the roots, so that conditions are favorable for continued active movement of salt into the upward conducting tissues. As we have seen, such a view does not imply any proportionality between salt absorbed and translocated and water transpired. For purposes of plant nutrition a very limited transpiration could suffice.

There is no inconsistency in recognizing that conditions occur under which absorption and movement of water may have a role in salt absorption and movement, and at the same time have clearly in view that

the relation between salt absorption and water absorption is not an indispensable one. The experiments on barley plants as described earlier in this lecture afford an illustrative case for the latter statement.

In an active root system an upward moving salt solution may be much more concentrated than the dilute culture solution, if only a small amount of water is being absorbed. When a large amount of water is being absorbed the internal salt solution will undergo more or less dilution. But in both cases metabolic factors are involved. Due appreciation must be accorded to movement of salt under the influence of root pressure, under some conditions, especially in young herbaceous plants.

The emphasis I have given to the activities of living cells is, I think needed, but this does not mean that salt can not be absorbed and translocated through injured or dead roots by a wick-like process. This is an interesting point with reference to plants growing in alkali soils in which some of the roots may become injured by high salt concentrations or by alkalinity and the factors associated therewith.

Finally, I may remark that although our knowledge of salt movement in the plant is still inadequate, analysis of existing evidence may permit a closer approximation to an understanding of these phenomena than has been available in the past. Most important of all, it seems, is recognition of the fact that the apparent simplicity of upward movement and distribution of salt in plants may be illusory. In this aspect of plant nutrition, it may be repeated, the dynamic and integrated character of the whole system is again emphasized.

REFERENCES:-

ARNON, D. I., STOUT, P. R. and SIPOS, F. Radioactive phosphorus as an indicator of phosphorus absorption of tomato fruits at various stages of development. Amer. J. Bot. 27: 791-798, 1940.

BARANETSKY, J. Untersuchungen über die Periodicität des Blutens der krautartigen Pflanzen und deren Ursachen. Abhandl. Naturf. Gesellsch. Halle 13: 1-64, 1877.

BROYER, T. C. and HOAGLAND, D. R. Metabolic activities of roots and their bearing on the relation of upward movement of salts and water in plants. American Journal of Botany 30: 261-273, 1943.

CLEMENTS, HARRY F. and ENGARD, CHARLES J. Upward movement of inorganic solutes as affected by a girdle. Plant Physiology 13: 103-122, 1938.

CRAFTS, A. S. and BROYER, T. C. Migration of salts and water into xylem of the roots of higher plants. American Journal of Botany 25: 529-535, 1938.

CURTIS, O. F. Translocation in plants. McGraw-Hill, New York, 1935.

ESAU, KATHERINE. Phloem anatomy of tobacco affected with curly top and mosaic. Hilgardia 13: 427-470, 1941.

GAUCH, H. G. and EATON, F. M. Effect of saline substrate on hourly levels of carbohydrates and inorganic constituents of barley plants. Plant Physiol. 17: 347-365, 1942.

GROSSENBACHER, KARL A. Diurnal fluctuation in root pressure. Plant Physiology 13: 669-676, 1938.

—— ——. Autonomic cycle of rate of exudation of plants. Amer. J. Bot. 26: 107-109, 1939.

HOAGLAND, D. R. Absorption of ions by plants. Soil Sci. 16: 225-246, 1923.

—— —— and BROYER, T. C. Accumulation of salt and permeability in plant cells. Jour. Gen. Physiol. 25: 865-880, 1942.

MASON, T. G. and PHILLIS, E. The migration of solutes. Botanical Review 3: 47-71, 1937.

—— ——, —— ——. Concerning the upward movement of soil solutes. Annals Bot. N. S. 4: 765-771, 1940.

PHILLIS, E. and MASON, T. G. The effect of ringing on the upward movement of solutes from the root. The effect of ringing and of transpiration on mineral uptake. Memoirs of the Cotton Research Station, Trinidad, Series B, No. 13: 635-650, 1940.

SKOOG, F., BROYER, T. C. and GROSSENBACHER, K. A. Effects of auxin on rates, periodicity, and osmotic relations in exudation. American Journal of Botany 25: 749-759, 1938.

STEWARD, F. C. Mineral nutrition of plants. Annual Review Biochemistry IV: 519-544, 1935.

—— ——, PREVOT, P. and HARRISON, J. A. The absorption and accumulation of rubidium bromide by barley plants. The localization in the root of cation accumulation and of transfer to the shoot. Plant Physiol. 17: 411-421, 1942.

STOUT, P. R. and HOAGLAND, D. R. Upward and lateral move-
ment of salt in certain plants as indicated by radioactive
isotopes of potassium, sodium and phosphorus absorbed by
roots. American Journal of Botany 26: 320-324, 1939.

WHITE, P. R. "Vegetable Dynamicks" and plant tissue cultures.
Plant Physiol. 17: 153-164, 1942.

THE GROWTH OF PLANTS IN ARTIFICIAL MEDIA IN RELATION TO THE STUDY OF PLANT NUTRITION

In the preceding lectures I have frequently referred to experiments in which plants are grown in culture solutions by the so-called water-culture method. In the present lecture I shall consider this method more specifically and with reference to its role in aiding in the elucidation of some general problems of plant nutrition. Knowledge has long been available that at least most kinds of higher plants that normally inhabit the soil, can be grown in a simple solution of nutrient salts. The earliest scientific record of what might be regarded as a crude water-culture experiment was published in 1699 by WOOD-WARD who grew plants in rain and river water with and without the addition of a little soil. The principles of plant nutrition were shrouded in obscurity in those days and WOODWARD's technique did not lead to any fruitful consequences. With the later clarification of the basic concepts of plant nutrition systematic work with nutrient solutions was carried on by SACHS and KNOP about 1860. Despite its long history, the water-culture method and its close ally, the pure sand-culture, remain of great service as tools for research in plant nutrition. Techniques have been extended and new methods of studying plants grown under conditions of nutrient solution control have been introduced. Advances in biochemistry open a wider

field for exploration of the effects of nutrient elements on the metabolism of the plant.

In a recent period artificial culture methods (water-culture, "hydroponics", sand-culture, gravel-culture) have attracted an extraordinary amount of attention as a means not merely to study general scientific principles of plant nutrition, but also to produce crops commercially. Some members of the general public seemed to gain the impression that a revolutionary development had taken place and that soon we could dispense with soil as medium for crop growth on a large scale. Many persons also thought, to judge from their letters of inquiry, that plants would grow without light, or in very feeble light, if only the right formula for a nutrient solution were available. But I shall not detain you with the history of this popular exploitation of the water-culture method. I need say only that, save in unusual situations, artificial culture methods including water, sand, and gravel cultures, are at present applicable in a commercial way only to expensive greenhouse crops. Even then practical and economic factors should receive critical consideration before the adoption of a novel technique can be justified.

The fact that plants that normally grow in soil can be grown in a purely inorganic medium has a rather far reaching significance scientifically, more now than formerly, since so many species have been shown to be adaptable to a water-culture medium. Numerous kinds of plants have been grown in this way through a reproductive cycle. We can, therefore, conclude that no unknown factor is present in the soil which is fundamentally indispensable for the growth of plants, at least of those represented by many agricultural species and some others. Evidently the organic matter of the soil so often stressed in considerations of soil fertility, is not directly essential from the point of view of the nutrition of the crop plant

itself, however important it is for secondary reasons. Some discussion has taken place with reference to the value of vitamin substances, especially vitamin B_1, added to the culture medium, but there seems to be fairly general agreement now that additions of vitamins to a nutrient solution do not have a significantly beneficial effect, except possibly under some exceptional circumstances. (*See plates 20, 21, and 22*).

Of course the water cultures as ordinarily conducted are not sterile and the argument might be advanced that microorganisms present produce growth substances necessary for the green plant. This seems improbable. Relatively few organisms develop in the inorganic culture solution, unless considerable organic matter enters the solution through liberation by dying roots. Great difficulty is found in obtaining direct evidence on this question by growing plants under sterile conditions, but cucurbit plants have been developed in this way in some of the experiments of BARKER and BROYER (1942), probably for a sufficient period of time to indicate that microorganisms played no indispensable role in the nutrition of the higher plant. In brief—as we should expect—the plants under discussion may be regarded as complete synthetic units under normal conditions for growth.

In making these remarks I do not forget that some at least of the same vitamin substances essential for animals are also essential for plants. The work on the culture of excised root tips shows that for vitamin B_1—possibly for other vitamins. In fact I do not think that it would be too violent an assumption that most or all the vitamins needed by animals, or their precursors, may have a role in plant growth and metabolism. The plant does not synthesize these substances merely for the benefit of the animal, but the important point at the moment is that it does synthesize them.

Conditions for the Growth of Plants in Artificial Cultures: — Granted that plants can grow in a solution of simple inorganic salts, it is then necessary to examine the factors of the solution environment that must be favorably controlled to insure the growth of the plant according to some preconceived idea of a proper rate of development for the prevailing climatic environment. In a previous lecture the suggestion was advanced that it is not possible to designate some one particular combination of salts as constituting a "best" nutrient solution under all conditions. Among other points, the rapid absorption of nutrient ions by growing plants and the ensuing change in composition of a nutrient solution were stressed as complicating factors. We have in fact to deal in solution cultures as in soils with a question of "supplying" power. Obviously, even if the initial composition of the nutrient solution is the same, plant growth over an extended period of time may differ greatly depending upon the initial volume of solution or on the number of times the solution is changed. In early researches on salt relations of plants investigated by water culture technique there was a tendency to overlook this consideration.

One of the requirements of the water-culture technique is to regulate the initial volume or the number of changes of solution so that no nutrient ions fall below a critical level of concentration unless it is the purpose to induce a specific deficiency or to study interrelations of ions without regard to maintained supply. Very low concentrations, at least of mobile ions like potassium, may suffice, if because either of a large initial volume of solution or of frequent changes, the solution never reaches a critical state of depletion. In general, absorption curves for nutrient ions show that relative to concentration, a much greater proportion of ions is absorbed from a dilute solution than from a more concentrated one.

The upper limit of the most favorable range of total salt concentration is not very high for many kinds of plants, perhaps about 2 or $2\frac{1}{2}$ atmospheres equivalent osmotic pressure in some cases. Therefore, if a large amount of nutrient salt is needed, over a given experimental period, for the growth of a given number of plants, one cannot simply increase the concentration in order to provide at the beginning of the experiment the entire supply of nutrient salts in a small volume of solution. The objective would have to be accomplished by sufficiently increasing the total *volume* of solution at a favorable initial level of concentration, assuming that it is not desired to add more nutrient salts from time to time, or to change solutions. What constitutes a critically low concentration of an ion at a particular period depends upon many factors, including the type of growth the plant makes. Wheat plants, for example, can absorb enough phosphate within a few weeks to insure a reasonably adequate supply stored in the plant for the remainder of the growth cycle. The tomato plant with its indeterminate growth presents a different problem.

In view of these, as well as many other complications, to decide whether or not a particular salt balance *per se* is favorable or unfavorable, is indeed very difficult. We can scarcely doubt that unfavorable salt balances can be produced in a nutrient solution irrespective of the sufficiency of total nutrient supply, but it appears that plants of similar size and similar general characteristics can be grown in solutions of varying salt proportions over a rather wide range of salt proportions, if the supplying power of the medium remains adequate. This is not surprising when we remember that plants may thrive in different soils, not alike in soil solution composition or in proportions of bases held on soil colloids. Further, general observation shows that plants of different species, with different physiological requirements, may thrive in the

same good soil; or often in the same nutrient solution.

It can be argued that every change in composition of a nutrient solution, however slight, might produce some changes in relative absorption of nutrients by the plant and in yield. This could be granted, at the
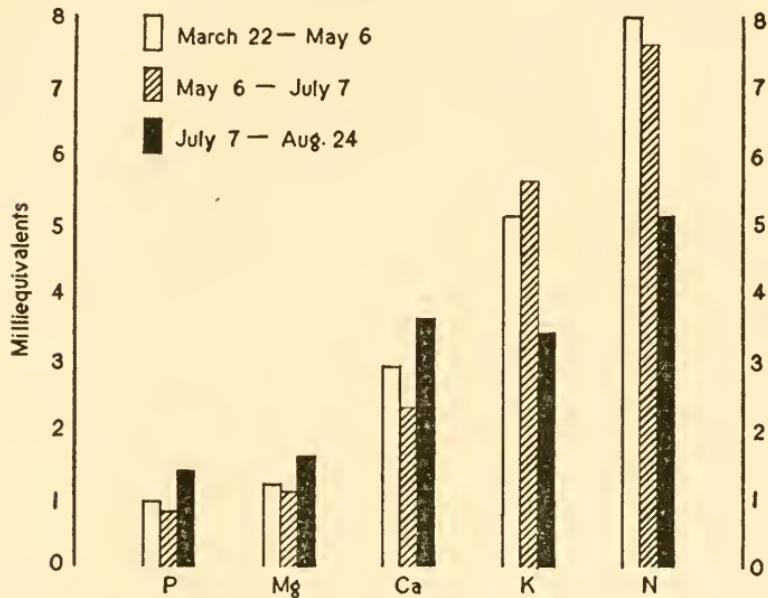
TEXTFIGURE 31. — Average daily absorption of nutrient elements from an aerated solution by a tomato plant, at various stages of growth, including fruiting stage. Quantitatively, nitrogen is of dominant importance; then follows potassium. The absorption of calcium was, however, slightly higher than that of potassium in the fruit stage of growth. (From ARNON and HOAGLAND, 1940).

same time suggesting that it might not be worth while, or even feasible, to establish small statistically valid differences in yield at the expense of a large amount of labor. The results, in any event, would be different for every different climatic complex. On the other hand, the inorganic composition of a plant

may be greatly influenced by the so-called luxury absorption of certain elements—that is, the absorption of increments of an element that do not produce any increase in weight of the plant, and this luxury absorption is dependent in part on solution composition. These general statements are not intended to discount the possibility that in special cases ionic relations may require careful adjustment. Shive has pointed out the importance of an interrelation between concentrations of iron and manganese in culture solutions, aside from the provision of an adequate total supply of these nutrients. Even so, no exact ratio is imperative.

The question of plant composition in relation to the nutrient solution is extremely complex. Ionic interrelations in absorption are important. Some of them are easily demonstrable even in dilute solutions when solutions are varied considerably in composition. As an illustration, with a given concentration range for calcium and magnesium in a nutrient solution, an increasing concentration of potassium will usually decrease absorption of these ions, per unit of plant growth, and so influence the percentage composition of the plant with reference to potassium, calcium and magnesium, a question to be discussed more fully in another lecture. Reciprocal relations between phosphate and nitrate ions have frequently been reported and have been met with in our own experiments. COLLANDER (1941) suggests that some ions closely related chemically act almost like isotopes in their absorption inter-relations. The complexities already mentioned are greatly augmented through the differential behavior of different species of plants growing in the same nutrient environment. COLLANDER's recent investigation gives some of the best evidence of this differential selective uptake of ions by plants of different species, growing in the same nutrient solution.

There is another concept which may be employed in the selection of salt proportions for a nutrient
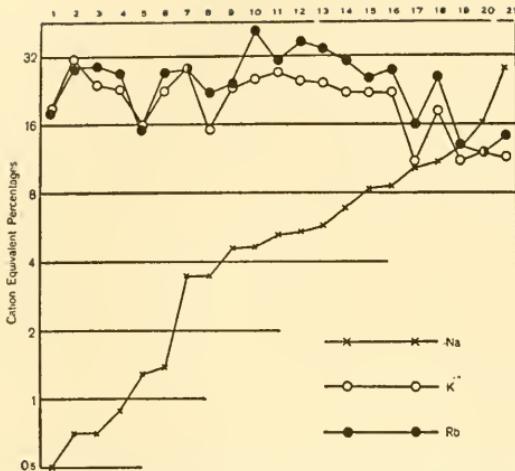
FIG. 1. Equivalent percentage of Na, K, and Rb in plants cultivated in solution III containing these cations in equivalent amounts. The plants are arranged according to increasing Na content.

1. Fagopyrum
2. Zea
3. Helianthus
4. Chenopodium
5. Salsola
6. Pisum
7. Nicotiana
8. Solanum
9. Spinacia
10. Avena
11. Aster
12. Papaver
13. Lactuca
14. *Plantago lanceolata*
15. Melilotus
16. Vicia
17. *Atriplex litorale*
18. Sinapis
19. Salicornia
20. *Plantago maritima*
21. *Atriplex hortense*

TEXTFIGURE 32. — Illustrating selective absorption of different cations by different species of plants growing in same nutrient medium. (From COLLANDER, 1941).

solution—that of what might be termed "economical" utilization of nutrients. By this is meant the prep- aration of a solution from which the plant will absorb the various ions in the same proportions as they are originally present in the solution. This is a goal that is seldom possible of achievement, because of the dynamic nature of the plant system and the varied inter-relations between ions in absorption, also because of the effects on absorption of nutrients of the physio- logical age of the plant. Yet we have had some success in preparing a nutrient solution along this line of approach for the growth of tomato plants (ARNON and HOAGLAND, 1940). It is scarcely necessary to say that the adjustment was only a rough one, but still it was useful as a practical method of managing the culture solution.

In previous lectures several observations on hydro- gen ion effects in nutrient solutions have been made. Something more on this point should be added now. None of our experiments suggest that plant growth, at least of any of the numerous species studied, is notably influenced by small variations in the pH of the culture media. In fact there seems to be a rela- tively wide range of hydrogen ion concentrations over which this factor *per se* has no highly significant effect on the plant. To isolate the hydrogen-ion va- riable under ordinary conditions of plant growth is not easy and perhaps never completely effected, even with more or less controlled artificial cultures. Thus, at a relatively high pH, plants grown in a nutrient solution may become chlorotic when inorganic iron and manganese salts supply these elements for the reason that precipitations may occur.

Considerable importance can be attached to the inter-relations between hydrogen ion and calcium ion concentrations. An example is found in some recent experiments conducted in our laboratories by ARNON and his coworkers (1942). It was desired to deter-

mine pH effects over a range of reactions. If a full nutrient solution were employed for this purpose, precipitation of calcium phosphate, or other salts, would take place at markedly alkaline reactions and so the general composition of the solution would not be the same over the whole range of pH. The basic solution was therefore regulated in its composition in such a way that all the salts remained in solution at the highest pH value it was sought to study. Also, to assure a supply of iron and manganese at alkaline reactions, these metals were furnished as synthetic humates. In other words, insofar as this is feasible, hydrogen ion concentration was made the chief variable throughout the entire set of cultures in certain of the series of experiments. The plants were grown in large volumes of nutrient solution, thoroughly stirred by aeration, so that within limits the desired hydrogen-ion concentration was maintained at root surfaces. Adjustments of reaction were also made as necessary, by addition of acid or alkali.

For all species of plants investigated, a pH maintained at 3.0 in the culture solution was markedly injurious, whatever other modifications were made in the solution. Just above the range of acid reaction that could not be tolerated by the plant the effect of hydrogen-ion concentration could be significantly influenced by the calcium ion concentration. In the weakly acid (pH 6) or in the slightly alkaline solutions no pronounced effect of varying calcium concentrations was apparent within a considerable concentration range. At the more acid reactions, however, the growth and vigor of the plants were superior at a high calcium concentration, as compared with a relatively low one. These relations have an interesting bearing on some of the problems of acid soils. In mineral soils a high hydrogen-ion concentration is likely to be accompanied by a low supplying power for calcium where, if the water culture experiments are

applicable, an especially high calcium concentration would be desirable.

In the course of the investigation now under discussion, nutrient absorption studies were carried out, with several suggestive results. Some of these experiments consisted in growing plants for a suitable period under favorable conditions and then subjecting the plants for short periods to solutions of various pH values. At the most acid reactions Ca was not absorbed by the plants and, in fact, loss of this element occurred from the roots to the solution. From solutions of otherwise similar composition those with the higher pH values permitted more calcium absorption to occur. On the other hand, at markedly alkaline reactions, little or no phosphate was absorbed. The full explanation of this effect is not now at hand, although several suggestions are under examination. (*See plate 23 and textfig. 33*).

As a special feature of nutrient solution technique it is well to keep in mind certain characteristics of the two forms of nitrogen that may be supplied, nitrate nitrogen and ammonia nitrogen. The ammonia nitrogen is absorbed as a rule so much faster than the associated anions that the tendency is for the solution to increase in acidity; the converse is generally, although not invariably, true of the nitrate ion. As TRELEASE and TRELEASE (1935) have pointed out, a convenient rough automatic control of pH of a solution during plant growth can sometimes be gained physiologically by supplying a suitable mixture of ammonia and nitrate nitrogen. We have had some success with this method in culture experiments on tomato plants.

Aeration of Nutrient Solutions: — With this brief survey of nutrient solutions with respect to their content of ions before us, permit me to return once more to the question of the aeration of roots. The potentiality for excellent growth and yield of fruit by

tomato plants growing in a culture solution is dependent on a liberal supply of oxygen to the roots and upon prevention of too high CO_2 concentration in the root environment. This objective may be achieved by the rapid passage of a current of air around the roots of each plant. A sand culture flushed daily with
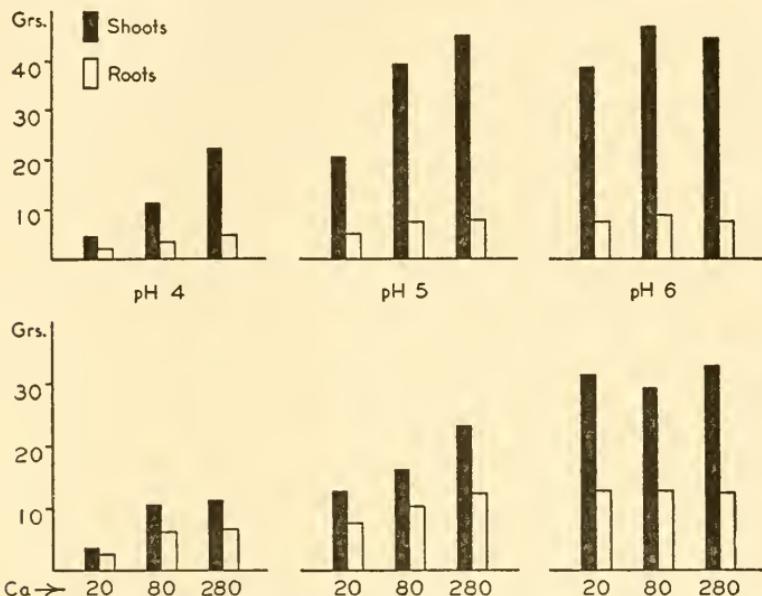
TEXTFIGURE 33. — Diagrammatic illustration of yields of roots and shoots of tomato plants grown at several pH values of nutrient solutions and with variation in calcium concentrations. Calcium concentrations in parts per million of solution. (From ARNON and JOHNSON, 1942).

nutrient solution likewise provides a suitably aerated medium. Our specially aerated water cultures produced plants of somewhat larger yield of fruit than we obtained from even an exceptionally favorable and heavily fertilized soil, when cultures were arranged side by side in the greenhouse.* The assump-

*The orders of magnitude of yields were, however, the same. It may be noted also that a porous bed superimposed over a tank of culture solution affords good, although not necessarily optimal, aeration.

tion is not unwarranted, in view also of evidence from the field, that frequently soil aeration constitutes a limiting factor for the growth of some types of crops and that it may be an ecological factor of significance.

Not only is the absorption of nutrients accelerated by the vigorous aeration of the culture solution in which tomato plants are grown, but there also exists a relation between aeration of the solution—that is, the introduction of oxygen and the removal of carbon dioxide—and the absorption of water. On hot days we and others have observed that tomato plants quickly show evidence of wilting if the aeration of the culture solution is stopped, or even markedly diminished. Whether this is merely a matter of permeability changes in root cells or whether some active transport of water is possible is not a finally settled question, but I argued in an earlier lecture that permeability is closely associated with metabolism. (*See plates 24 and 25*).

Many of these remarks have as their basis the results of experiments conducted with tomato plants. Valuable as the tomato is as a test plant, to draw too broad conclusions from this one species would be most unwise. For the same type of aeration that is so beneficial to the growth of the tomato plant is injurious to some other kinds of plants. The rice plant is an example of a plant that may be injured by too high aeration of the root system. The willow is another. Of course, knowing the conditions under which these plants can grow out-doors this response is not surprising. The question merits some additional comments. VLAMIS (1941) carried on an investigation in Berkeley of salt absorption by the rice plant. In the first place, he learned that the excised roots of barley, tomato and rice plants did not behave very differently with respect to effects of oxygen on salt

absorption, as tested by bromide ions, but the intact plants did not react alike. Rice plants can absorb salt and grow satisfactorily when the roots are placed in an anaerobic or nearly anaerobic environment. The conclusion seems to be that necessary oxygen required by the roots of the rice plant can be translocated from the shoot.* To a somewhat lesser degree this would be true of the barley plant. According to the studies of VLAMIS, a high rate of aeration of the culture medium, while it may temporarily accelerate salt absorption by the rice plant, soon produces injured root tips.

Another factor to be taken into account in a discussion of nutrient solutions or other root media is that of temperature. It was mentioned in a previous lecture that short period studies on the absorption and accumulation of inorganic solutes by excised roots of barley plants, or by intact plants, disclose a high temperature coefficient for this process, within certain ranges of temperature. In longer time periods the temperature question becomes much more complex, since the whole plant system is undergoing change and development. Furthermore, rates of movement of solutes from the root to the top of the plant and of the transport to the root of carbohydrate or of growth substances must also govern the activities of roots and the absorption and upward transport of solutes, as I have already attempted to show. There seems to be comparatively little evidence on record of root temperature effects on plant growth from experiments conducted under controlled conditions by water-culture technique. A series of systematic studies has therefore been initiated on crop plants of different species to be grown under the aerial environment of a greenhouse at different seasons, with the nutrient solutions

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*There is some evidence that plants of this character possess anatomical adaptations facilitating the internal aeration of roots.

subjected to different temperatures and degrees of aeration. Some results are now available, sufficient to indicate that root temperature responses vary pro- foundly depending on the kind of plant studied. (*See plate 26*).

Aeration is a factor interrelated with temperature. At higher temperatures the rate of oxidation of carbo- hydrate is increased when abundant oxygen is avail- able to the roots. This may lead to eventual injury. Climatic conditions affecting the growth of the shoot and photosynthesis also modify responses of the plant to varied root temperatures. In experiments with tomato plants developed in artificial culture solutions in the Berkeley greenhouses we have not found it necessary or desirable to heat the nutrient solutions. A favorable air temperature seems to result in suit- able adjustment of the temperature of the nutrient solutions, although it is not unreasonable to suppose that under natural or agricultural conditions the soil may sometimes be too cold early in the growing season to permit rapid root growth and solute ab- sorption. There are practical observations to support this assumption.

Climatic Factors and Growth of Plants in Nutrient Solutions: — In all this discussion of nutrient solu- tions and their control, I have not yet given enough emphasis to the climatic factors, but you may recall that I said something in the first lecture about cul- tures carried on in controlled chambers. Most of the investigational work has to be conducted in green- houses, without control of light. The climatic factors assume great importance in the study of plants grown to the fruiting stage. Mobilization of inorganic nu- trients in growing tomato fruits profoundly affects the nutrition of the plant and its relation to the culture solution. Nitrogen-carbohydrate relations in- fluencing fruit development depend on light-tempera-

ture conditions. In summer in Berkeley the environment has been so favorable (adequate light and relatively low night temperatures, preventing excessive respiration) for the operation of these factors in growing tomato plants that extremely fruitful plants have been produced, even when nitrogen was supplied to the full extent of the plant's capacity to absorb nitrogen. Under another climatic complex a different conclusion might be reached. The indispensability of appraising the mineral nutrition of the plant with reference to carbohydrate synthesis is well exemplified by the investigation of NIGHTINGALE (1942) in Hawaii on the pineapple plant and that of CLEMENTS on sugar cane. These relations are of importance even though modern research reveals the significance of hormone factors in the initiation of flower buds and in determining other physiological processes.

Nutrition of Plants in Soil and in Artificial Culture Solutions: — I have surveyed some of the physiological aspects of the inorganic nutrition of plants as they are offered for examination under the controlled conditions of artificial culture technique. I do not think that there is reasonable doubt that by experiments of this kind we do gain an insight into certain plant processes that is of great service in understanding the nature of the soil-plant system. Yet the role of soil colloids in plant nutrition intrudes when we attempt a too direct and simple application of the results of artificial culture experiments to the growth of plants in a soil medium. After all, the union of the absorbing root organ and the soil colloid is a very close one and contact effects of one kind or another are inevitable. In an aerated solution or in a sand culture through which a nutrient solution is flushed from time to time the nutrient ions are brought to the roots. In the soil the roots explore the soil, and develop enormous areas of root surface under favor-

able conditions. Therefore the plant forms innumerable points of intimate contact with colloidal soil particles.

Some of the contact effects between root and soil colloid may be interpreted in terms of soil solution phenomena, but several of my colleagues, especially JENNY and OVERSTREET (1939), have recently proposed that a more direct contact relation operates.* They have sought to study this question by a combination of a water culture and colloid system. Purified clay colloids with different proportions of adsorbed ions were prepared and suspended in water or in nutrient salt solutions. Excised barley roots of high salt absorbing capacity, like those already described, were immersed in the suspensions. In many instances the movement of ions between root and colloid was investigated usefully by radioactive tracer methods, particularly with reference to cation movement.

The theory underlying the explanation of some of the results is that ions adsorbed on colloidal surfaces may undergo surface migration, one ion exchanging for another within the oscillation volumes of the ions. There is direct evidence that this can happen in a soil colloid system. JENNY and OVERSTREET go further and believe that such an ion exchange can occur between the root and soil colloid and form an efficient mechanism for the removal by the plant of ions adsorbed on the soil colloids, through exchange of metabolically produced hydrogen ions for calcium, magnesium, potassium, or other ions held by the soil colloids. The chief immediate nutrient reserve for some ions is found in these adsorbed ions. Nevertheless, the soil solution mechanism by no means loses its interest because of the possibilities of contact absorption. Certain ions, notably nitrate ions, are not adsorbed to any important degree by the soil colloids

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*An early suggestion was made by COMBER (1922) for a modification of the soil solution hypothesis.

and anions present in the soil solution must necessarily be accompanied by equivalent quantities of

$$\boxed{\text{Root}} \rightarrow \underbrace{m(H_2CO_3)}_{\text{In soil solution}} + \boxed{\text{Clay} \begin{array}{c} Ca \\ \\ Ca \end{array}} \rightarrow$$

$$\boxed{\text{Clay} \begin{array}{c} H \\ H \\ Ca \end{array}} + \underbrace{Ca(HCO_3)_2 + (m-2)(H_2CO_3)}_{\text{In soil solution}}$$

Contact intake

Contact depletion
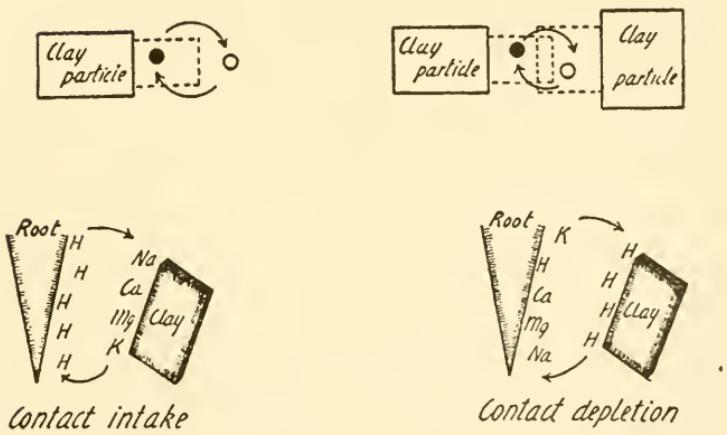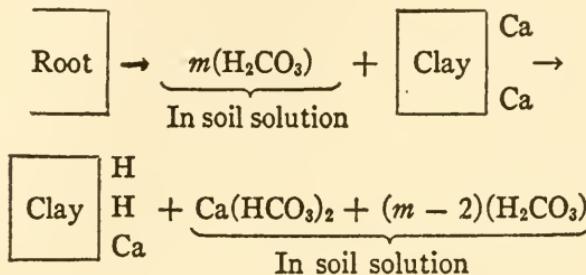
TEXTFIGURE 34. — Top: soil solution mechanism in utilization of absorbed ions. Middle: contact exchange of ions in overlapping oscillation volume of clay particles. Bottom: exchange of ions between root and clay particles. (From JENNY and OVERSTREET, 1939).

cations. Further consideration of this general point will be given in a discussion of problems of potassium nutrition.

The active transport of ions in the roots is not rendered less important by adopting the view that contact modes of ion absorption have a role in plant nutrition. Whatever the first step in the removal of ions from the soil may be, the continuance of the absorption demands that ions be rapidly removed from root surfaces and this is accomplished by the processes of active ion transport in the plant. The attainment of a true equilibrium condition in the root-soil system does not occur. Metabolically induced ion movement, most clearly demonstrated by experiments with artificial culture technique, has significant consequences for the explanation of the availability of nutrient ions present in the soil. We have here one reason why the laboratory determination of the availability of potassium, phosphate and other ions is fraught with so much difficulty.

Other Uses of Artificial Culture Methods: — There are still other problems, the solution of which may be aided by the use of artificial culture methods. One of these is concerned with the quality of plant products from the point of view of human and animal nutrition, concerning which I have already said something in another lecture. As in the general investigation of plant nutrition so too in this still more intricate investigation of soil, plant, and animal, one cannot hope to lay a secure foundation by attacking the whole system at once. Much can be gained by first attempting to assess the degree to which the plant is subject to change in its composition through modification of controlled nutrient media and of climatic factors. Clearly the methods of solution and sand culture offer one of the means of approach. Under what conditions can the content of the plant in calcium, phosphorus, iron, manganese, cobalt or other chemical elements of significance in animal nutrition be increased? What is the influence of nutrient elements absorbed by the plant on its ability to synthesize vitamin substances?

To answer questions like these demands the application of all the tools of plant nutrition, one of which is the method of artificial culture. I have already spoken of the Federal laboratory at Cornell University established to study problems of this kind.

In my first lecture I referred briefly to a problem of concern to my own part of the United States—the problem of alkali soils. Here too we must deal with a multiphase system. One of the efforts must be to understand the effects of the alkali salt on the plant, going beyond the ordinary concentrations and ionic balances of nutrient solutions. At the present time sand culture methods are in use at the Federal Salinity Laboratory at Riverside, California, for ascertaining the tolerances of different species of plants to varied concentrations of sodium salts. Installations are located in three climatic areas; namely, Riverside, the Coachella Valley and on the coast at La Jolla. These sites differ greatly in light, temperature and humidity.

There are still other uses for controlled artificial culture methods of growing plants, although some of them have not yet received much general attention. Considerable interest is occasionally manifested by students of entomology in the possibilities of controlling the nutrition of the plant and perhaps modifying in a known way the ability of the plant to resist insect attacks, or at least to explain a little more clearly certain observations on plant-insect relations. Some experimental work has been initiated.

Plant pathologists likewise may possibly find advantages in a method for controlling the inorganic nutrition of the plant. Sporadic observations have been recorded of interrelations between the nutritional status of a plant and its susceptibility to injury by certain pathogenic organisms. Wheat plants grown in a medium very low in silica often become highly susceptible to fungus attack. SPENCER (1942) has suggested opportunities for the investigation of the controlled nitrogen nutrition of the plant in relation

to the increase of virus protein in an infected plant. Perhaps also the student of plant anatomy and morphology may find that he can cooperate at times with workers who have experience in managing culture solutions.

One of the outstanding values of controlled culture technique, no doubt, will eventually be found in the studies biochemists can make on plants subjected to controlled environments. I observed in the first lecture that biochemistry applied to higher plants has not received nearly the degree of effort it deserves, despite important individual achievements. I have the impression that artificial culture methods, including climatic control in appropriate cases, as well as control of nutrient media, will render important service to plant biochemists.

Concluding Statement: — In these lectures as a whole the attempt has been made to survey some of the problems of plant nutrition and to give a general perspective of this field of inquiry. Of the limitations of the discussions I am keenly aware. Plant nutrition is not of itself a science. Its study rests on the application of other sciences to a vastly complex system. Progress is slow and laborious. There does not usually exist the possibility of setting down in the precise and elegant terms of the physical scientist the course of events in the growing plant.

The practical objectives form a compelling incentive for a worker in an agricultural experiment station. Our industrial civilization rests on ability to produce all the food we need by the efforts of a small fraction of our total number of workers. This can be accomplished only because of advances in agricultural arts and sciences. Certainly one of the important scientific foundations of modern crop production is knowledge of plant nutrition. Still I suspect that, as in many other subjects, we are led on to continued research because of curiosity; the desire to find out what plants

do and how the system operates. If I have succeeded in these lectures in convincing you that research in Plant Nutrition has made a useful beginning in satisfying this curiosity, I shall be content.

REFERENCES:-

ARNON, D. I. and HOAGLAND, D. R. A comparison of water-culture and soil as media for crop production. Science 89: 512-514, 1939.

—— ——, —— ——. Crop production in artificial culture solutions and in soils with special reference to factors influencing yields and absorption of inorganic nutrients. Soil Science 50: 463-485, 1940.

—— ——, FRATZKE, W. E. and JOHNSON, C. M. Hydrogen ion concentration in relation to absorption of inorganic nutrients by higher plants. Plant Physiology 17: 515-524, 1942.

—— —— and JOHNSON, C. M. Influence of hydrogen ion concentration on the growth of high plants under controlled conditions. Plant Physiology 17: 525-539, 1942.

BARKER, H. A. and BROYER, T. C. Notes on the influence of microorganisms on growth of squash plants in water culture, with particular reference to manganese nutrition. Soil Science 53: 467-477, 1942.

COLLANDER, RUNAR. Selective absorption of cations by higher plants. Plant Physiology 16: 691-720, 1941.

COMBER, N. M. The availability of plant food. A modification of the present hypothesis. Journal Agricultural Science 12: 363-369, 1922.

HOAGLAND, D. R. and ARNON, D. I. Physiological aspects of availability of nutrients for plant growth. Soil Science 51: 431-444, 1941.

JENNY, H. and OVERSTREET, R. Cation interchange between plant roots and soil colloids. Soil Science 47: 257-272, 1939.

—— ——, —— ——. Surface migration of ions and contact exchange. Journal Physical Chemistry 43: 1185-1196, 1939.

NIGHTINGALE, G. T. Nitrate and carbohydrate reserves in relation to nitrogen nutrition of pineapple. Botanical Gazette 103: 409-456, 1942.

OVERSTREET, R. and JENNY, H. Studies pertaining to the cation mechanism of plants in soil. Proc. Soil Science Soc. of America 4: 125-130, 1939.

SPENCER, ERNEST L. Specific biological activity of tobacco vines as influenced by age of lesion and nitrogen supply. Plant Physiology 17: 210-222, 1942.

TRELEASE, S. F. and TRELEASE, HELEN M. Changes in hydrogen-ion concentration of culture solutions containing nitrate and ammonium nitrogen. Amer. Jour. Bot. 22: 520-542, 1935.

VLAMIS, JAMES. Studies on salt absorption by the rice plant. Thesis for Ph.D. degree, University of California, 1941.

SOME BIOCHEMICAL PROBLEMS ASSOCIATED WITH SALT ABSORPTION

Whenever salt is accumulated or actively transported by living cells, their metabolism is inevitably involved. An understanding of the mechanisms that result in salt movement (or the active movement of other solutes) must ultimately be found largely in the realm of biochemistry. It is the purpose of the present discussion to summarize several aspects of the problems of chemical processes occurring in plant tissues as salt is moving into or through the cells, or soon after the salt has been accumulated.

There is need at once for recognizing a difficulty in the interpretation of biochemical observations in terms of certain views on salt accumulation discussed in recent literature. That is, to decide whether or not a metabolic reaction taking place concomitantly with the entrance of salt into the cell has any causal relationship to the continued accumulation of the salt. For example, in roots and storage tissues (as represented by the potato tuber), the accumulation of potassium salt is usually accompanied by an increase in CO_2 production, with tissues placed in distilled water as a basis for comparison. One possible type of assumption is that a small amount of potassium or other ions first enters the protoplasm and stimulates metabolic reactions that lead to liberation of cellular energy available for the accumulation of salt against a potential gradient. On the other hand, the increase of CO_2 production could be merely the effect on metabol-

ism of the intake of the salt with the energy for this process provided by the respiration that would take place in the absence of potassium in the external solution. Some potassium absorbed during the growth period of the plant would of course already be present in the tissue and so could serve the indispensable purpose of this element in metabolism. A similar question would arise concerning any other ions.

Whatever view may be taken of this particular aspect of the phenomena, it is significant that in storage tissues and in roots the absorption of certain salts leads to an increase in respiration as denoted by CO_2 production or oxygen consumption. STEWARD suggests that ions or salts with the capacity to bring about this stimulation are most effective during their actual passage into the living cells.

One point should be made clear concerning the influence of salt absorption on respiration in view of recent discussions. There appears to be no disagreement among investigators in this field as to a salt effect on respiration by roots and storage tissues. The divergence of views has to do with the nature of the effect as it is related to the kinds of ions being absorbed. LUNDEGÅRDH and BURSTRÖM (1933) have elaborated a theory of anion respiration. This theory postulates that there exists in plant tissues a basic respiration and that this is augmented by a respiration stimulated by the absorption of anions (although secondarily influenced by cations). It is this anion respiration which provides energy for the accumulation of the anions. The cation is thought to be accumulated only as a consequence of the accumulation of the anions. The cations are conceived to be liberated at internal phases of the cell after they are first taken up by the protoplasm through a process of adsorption. Many of the studies on potato discs and on roots in this laboratory and in STEWARD's laboratory are not consistent with the hypothesis of a special anion respiration in the sense of LUNDEGÅRDH. It would not

be appropriate in this lecture, however, to undertake a controversial discussion. The results of the experiments to be considered may incidentally have a bearing on the theory outlined, but the main purpose is more general.

More sugar usually disappears from excised roots when potassium salts are absorbed than when absorption occurs from calcium salts and almost always respiration is accelerated more by potassium salts than by calcium salts.* Sugar concentration in the roots does not, within wide limits, determine directly the rate of respiration. One of the problems that awaits further investigation is concerned with the fate of sugar during root respiration and salt accumulation. Much more sugar may disappear from the roots than is accounted for by the CO_2 evolved. It is probable that part of the sugar is converted into polysaccharide form, but the direct evidence for this is not at present available. Appreciable amounts of starch are not found in these barley roots but another hexosan has been isolated.

Salt Absorption and Organic Acids: — A question of first importance in the study of the inorganic nutrition of plants in relation to biochemical processes is that of the effects of salt absorption on the organic acid system of the plant cell. By organic acids in this connection is meant the ether-soluble, non-volatile organic acids, particularly oxalic, succinic, malic and citric acids, and no doubt others entering into cycles of organic acid transformations.

Organic acids of this type are of great significance in the metabolism of plant cells and some of them have as one function a role in buffer systems of the

*In the absorption of ions from calcium solutions but little calcium is absorbed. With respect to absorption of ions by roots from calcium bromide solutions, the equivalent of bromide removed from solution exceed greatly those of calcium but more bromide is removed from a potassium salt than from a calcium salt.

cell sap. These acids, in association with inorganic bases, potassium, calcium, magnesium, and sodium, together with phosphate, amides, amino acids and other components constitute a buffer system which contributes to the maintenance in living plant cells of a hydrogen ion concentration held within fairly narrow limits, at least for many types of living cells of the plant. But the system is not merely a static one in which only simple chemical buffering determines the hydrogen-ion concentration. The cells are capable of responding to tendencies toward change of hydrogen-ion concentration caused by entering solutes through appropriate metabolic reactions that prevent wide fluctuations in hydrogen ion concentrations.

Early plant physiologists did not have the advantage of modern concepts of buffer systems and of hydrogen ion activities with appropriate techniques of study, but they were aware that organic acids are associated with the acid-base metabolism of plants. This is illustrated by the following quotation from PFEFFER:

> "By means of this self-regulatory power of increasing or decreasing the production of acids, an excessive formation of basic or alkaline substances may be compensated for, while on the other hand a dangerous accumulation may be avoided when neutralizing substances are produced in less than normal amount."

One view of an earlier period was that bases, particularly calcium, absorbed by plants prevent toxicity that would otherwise ensue from the metabolic production of organic acids, notably oxalic acid. DUNNE (1932) examined this supposition in our laboratory by means of experiments on buckwheat plants grown in controlled culture solutions. He came to the opposite conclusion; namely, that oxalic acid is synthesized in part in response to calcium absorbed by the plant. Recently similar conclusions have been drawn from experiments by other investigators on different plants. When nutrient solutions were varied in com-

position so as to bring about increasing calcium absorption, increasing concentrations of oxalic acid appeared in the plants. In certain instances a roughly stoichiometric relation was observed.
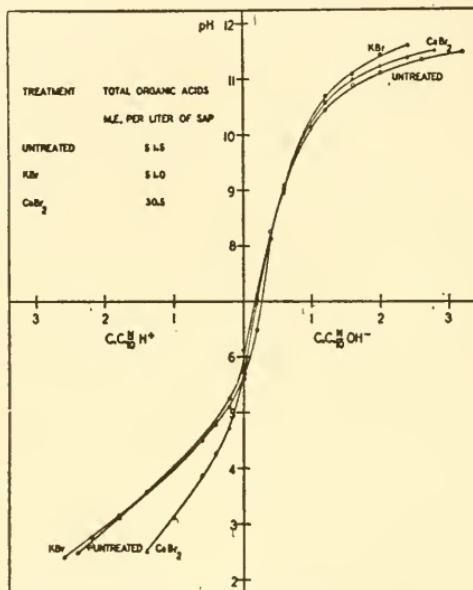
TEXTFIGURE 35. — Buffer contents of composite expressed saps as influenced by the nature of the salt supplied in the culture solution during solute absorption. In this particular experiment the equivalents of K and Br absorbed were approximately the same. Br was absorbed to a much greater extent than Ca. In this and the following figure the acid titration values reflect the organic acid contents of the sap, in agreement with direct analysis. (From HOAGLAND and BROYER, 1940).

Emphasis is needed for the role of potassium in sap buffer systems. Generally potassium is the chief inorganic base in equilibrium with organic acid systems. The supposition has sometimes been made that the absorption of calcium prevents the plant tissue

from becoming too acid. Actually it may happen that potassium deficiency leads to a slightly more acid reaction of the sap, despite relatively large calcium and magnesium absorption, a point discussed further in the next lecture.

Since so much information was at hand on the nature of the salt absorption process in barley roots, an investigation of their organic acid metabolism was considered useful and ULRICH (1940-41-42) undertook
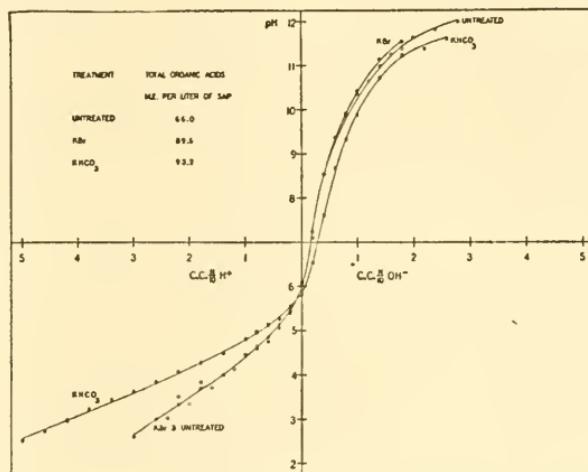
TEXTFIGURE 36. — Buffer contents of composite expressed saps as influenced by the nature of the salt supplied in the culture solution during solute absorption. The effects of KBr and KHCO₃. (From HOAGLAND and BROYER, 1940).

this. Many factors were studied, but of specific interest now are the experiments that showed that the total organic acids of the roots are subject to increases or decreases in concentration in the sap as a result of the unequal intake by the roots of cations or anions.

From previous discussion, it may be recalled that young roots initially low in potassium can absorb,

from a potassium salt solution, in an initial period of absorption, more equivalents of potassium than of even a mobile anion like bromide. The solution becomes more acid, although the increase in hydrogen ion concentration does not account for all the excess of potassium absorbed. The balance of cations and anions is in part maintained by entrance into the solutions of calcium and magnesium ions, possibly derived from protoplasmic or cell wall phases. From solutions of potassium sulphate, potassium ions have always been observed to enter the roots much more rapidly than the sulphate anions, which are absorbed extremely slowly. These selective absorptions of cations lead to increased organic acid synthesis by the tissue.

Decidedly striking is the effect observed when potassium is accumulated from a dilute solution of potassium bicarbonate. Under aerobic conditions potassium is absorbed as readily from the bicarbonate as from the bromide.* The entering potassium is approximately balanced by newly formed organic acids. If potassium moves inward accompanied by bicarbonate ions the latter are later broken down and the carbon dioxide given off. Bicarbonate ions do not appreciably accumulate in the cells. Some investigators would prefer to regard the absorption of potassium as a potassium-hydrogen ion exchange, accompanied by formation of organic acids by the roots. The net effect would be the same for either mechanism.

Less readily demonstrated but frequently significant are decreases in organic acid content of roots found when anions are absorbed in excess of cations. The anions are then replaced in the culture solution, directly or indirectly, by bicarbonate ions derived in part, it would appear, from oxidation of organic acids.

*Since bicarbonate ions are not considered to stimulate respiration, it is difficult to understand how potassium accumulation can be related to the theory of anion respiration under these conditions.

In the tissue, the proportion of organic acid to base is decreased and possibly by selective oxidation the relations of acids of different dissociation constants
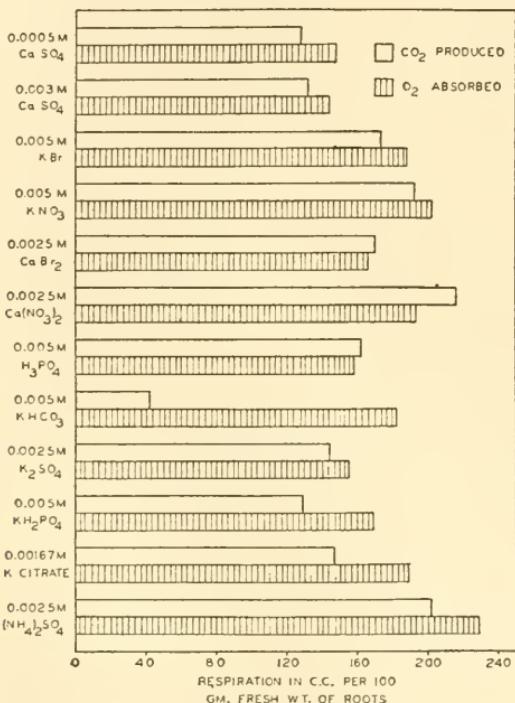
TEXTFIGURE 37. — Respiratory quotients (CO_2 produced/O_2 absorbed) of young barley roots as influenced by selective ion absorption. From K salts the K ions are often absorbed in greater equivalent amounts than the anion. Organic acid is synthesized and O_2 absorbed exceeds CO_2 produced. A very large disproportion is found when absorption occurs from a $KHCO_3$ solution. The bicarbonate ion does not accumulate appreciably in the roots. From some calcium salts the CO_2 produced exceeds the O_2 consumed, with a tendency for organic acid to decrease. (From ULRICH, 1941).

are altered. Excess absorption of anions is in general characteristic of roots absorbing from solutions of calcium salts with mobile anions like calcium bromide.

The general tendency is for the root cells to make metabolic adjustments in the direction of maintenance of hydrogen ion concentration in the sap within a limited range of values. Evidence for this can be gained only by observations on composite sap expressed from the tissues and this admittedly is a crude method. It is true also that there are appreciable variations of pH of the expressed sap under different physiological conditions. Nevertheless, considering the impossibility of obtaining for study sap in an unaltered state, and the great variety of conditions to which the root tissues have been subjected, the evidence for a certain stability of hydrogen ion concentration, provided that injury to the cells is not present, is noteworthy and must be regarded as of physiological significance.

In order to understand the effects on respiration of selective ion absorption, it is necessary to determine not only carbon dioxide produced, but also oxygen consumed and to take into account the respiratory quotient, CO_2/O_2. This has been done in the investigation of root metabolism just mentioned. An apparatus was employed through which air or other gas mixtures were circulated and in which oxygen could be kept at a desired tension through the automatic generation of oxygen by electrolysis. As oxygen was absorbed by the tissues, compensatory volumes of oxygen were produced. The carbon dioxide evolved was trapped in a sodium hydroxide solution. The values as a whole for respiratory quotients are in satisfactory accord with the view suggested with regard to organic acid metabolism and salt absorption. The entrance of basic ions without equivalent entrance of anions (unless of HCO_3 or OH ions) results in less carbon dioxide given off in respiration in relation to oxygen consumed— sugar is oxidized in part to organic acids. The excess absorption of anions accompanied by a decrease of organic acids leads to an increase of the respiratory

quotient, since organic acids initially present are being oxidized.

There is one important aspect of the phenomena for which our present information is deficient, a point well emphasized by the investigations of VICKERY and his associates. A true picture of the system would require that we understand not only changes in total
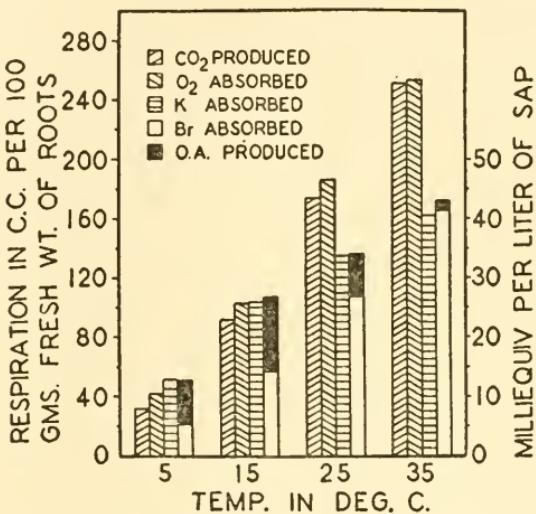
TEXTFIGURE 38. — Relationship of organic acid formation to respiration and to the absorption of potassium in excess of bromide ions. (The length of the solid black column and not the total height of the white plus black column is equivalent to the organic acids produced). (From ULRICH, 1941).

organic acid content of the tissues, but likewise in the percentages of individual acids, which may have different dissociation constants. In some cases a large proportion of the total acids have to be assigned simply to unknown acids. As far as the barley roots are concerned, most of the organic acid content is accounted for by malic $\begin{smallmatrix} COOH \\ CHOH \\ CH_2 \\ COOH \end{smallmatrix}$, oxalic $\begin{smallmatrix} COOH \\ COOH \end{smallmatrix}$ and citric

$$\text{acids } \underset{\substack{\text{CH}_2\\\text{COOH}}}{\overset{\substack{\text{COOH}\\\text{CH}_2}}{\text{HOC-COOH}}}, \text{ with malic acid greatly predominat-}$$

ing. When the total organic acids are increased by the absorption of potassium from the bicarbonate, most of the increase is in malic acid, according to the studies of ISAACS in this laboratory.

Metabolism in Relation to Absorption of Nitrogen: —Of special importance to the study of plant nutrition is the relation of the absorption of nitrogen containing components of a culture medium, nitrate or ammonia nitrogen, to the organic acid system. The fate of absorbed nitrogen is concerned with the whole nitrogen metabolism of the plant. The latter is a very large subject already outlined in a monograph by CHIBNALL (1939) and in the extensive contributions of VICKERY and his collaborators (1938-39-40-42), which makes elaborate discussion unnecessary. Several questions, however, warrant brief attention for our immediate purpose.

Nitrate ions can be stored in plant cells, sometimes in large quantity, but normally their ultimate fate is to be reduced. The nitrogen becomes part of nitrogen containing organic compounds; amides, amino acids, proteins and other nitrogenous products, in part unknown. While nitrate ions can be absorbed by plants in excess of bases absorbed, in considerable measure the nitrate ions enter with cations, commonly potassium ions. After the reduction of the nitrate a base remains, which tends to be neutralized by the formation of organic acids in the manner described. Suppose, however, that nitrogen is furnished to the plant in the form of ammonia nitrogen. Then the nitrogen is absorbed in cation form, or its equivalent, and the nitrogen is utilized in the plant for synthesis of organic nitrogen. The constitution of the cell sap is greatly modified from that of a plant receiving nitrate nitrogen. The content of organic acids is lower and as

CLARK (1936), in VICKERY's laboratory, has demonstrated in experiments on tomato plants, the qualitative composition of the organic acid mixture may be markedly altered. The proportion of unknown organic acids was much greater for the plants absorbing ammonia nitrogen than for those absorbing nitrate nitrogen.

VICKERY, PUCHER, WAKEMAN and LEAVENWORTH (1940), who have contributed so many results to research on the nitrogen and organic acid metabolism of higher plants, have pursued these studies with reference to the tobacco plant. The effect of increasing the proportion of ammonia to nitrate nitrogen in the culture solution produced a profound decrease in the concentrations of organic acids in all parts of the plant. Malic and citric acid were most sensitive to change in cultural conditions. It is noted that in certain treatments the plants were of the same size and appearance, yet were chemically very different as regards the organic acid constitution of the sap. The flexibility of plant metabolism is illustrated.

Plants growing under ordinary soil conditions are usually supplied nitrate as their chief source of nitrogen and consequently would have the organic acid metabolism characteristic of the absorption of bases in association with nitrate ions, followed by the reduction of the latter within the plant. Nevertheless, the capacity of the plant to respond metabolically to another source of nitrogen is of great interest to the effort to elucidate the kinds of reactions that can take place. Furthermore, there are some soil conditions under which ammonia nitrogen may become important, as in the growth of rice plants in a flooded soil.

In nutrient solution investigations the ammonium ion ordinarily can be completely substituted for the nitrate ion, but generally more precautions must be taken in controlling the solution when nitrogen is supplied as ammonia than when it is supplied as nitrate. The point has been made in a previous lecture that

the solutions made with ammonium nitrogen rapidly become more acid, for the reason that the ammonium ion (if ammonia is absorbed by another mechanism the result would be the same) is removed at a greater rate than the associated anions, sulphate and phosphate. The solution may eventually become so acid as to produce injury to the plant. Internally, the ammonia can react quickly with organic substrates. Whether they are sufficient in supply to take care of the ammonia absorbed depends ultimately on the synthesis of carbohydrates in the green parts of the plant. On the other hand, as already stated, nitrate can be stored, if necessary, in inert form. The adjustment of a plant to ammonia is, therefore, especially dependent on climatic environment as governing photosynthesis and respiration. ARNON (1937) found definite evidence of these interrelations in growing barley plants with the two sources of nitrogen at various seasons of the year under well controlled nutrient solution conditions.

Nitrate is utilized by plants only following its reduction, apparently through the stages of nitrite and ammonia. Knowledge of the mechanism and enzyme systems involved in nitrate reduction is still very meager. Light is not essential as a direct factor in its reduction. Excised barley roots and various other plant tissues can readily reduce nitrate in darkness. The normal course of nitrate reduction for some kinds of plants seems to proceed almost entirely in the root system. Light, of course, is indirectly an indispensable factor, in providing at some stage of the metabolic cycle photosynthetic substrates.

One feature of nitrate as a source of nitrogen is that it contains oxygen as well as nitrogen, and so is an effective oxidising agent. There are suggestions that this action is of some value under circumstances which limit the oxygen supply to roots.

Whatever the source of inorganic nitrogen, we are faced with the problem of the synthesis of amides and amino acids, preceding the synthesis of proteins or other complex nitrogen containing compounds. The two principal amides formed are asparagine $\begin{smallmatrix} COOH \\ CHNH_2 \\ CH_2 \\ CONH_2 \end{smallmatrix}$ and glutamine $\begin{smallmatrix} COOH \\ CHNH_2 \\ CH_2 \\ CH_2 \\ CONH_2 \end{smallmatrix}$. Earlier investigations comprehensively surveyed by CHIBNALL and by VICKERY developed the view that these amides are synthesized as a response to ammonia absorption and that they act as a means of detoxication of ammonia. Later the theory was advanced in Germany that plants with markedly acid reactions of the sap can accumulate ammonium organic acid compounds without injury and that plants may be roughly divided, on the basis of this distinction, into "amid" plants, with slightly acid reactions, and "ammonia" plants, with markedly acid reactions.

These general concepts have had value in the study of the nitrogen metabolism of plants, but the investigations of VICKERY and his associates, and those of CHIBNALL, have made us aware that there are more fundamental considerations to be examined, and that these rest on modern research in biochemistry. Much of this research has originated in the work of biochemists who have experimented with animal tissues, but in the study of many phases of metabolism a useful transfer of knowledge is possible in providing suggestions and guidance, in the present status of knowledge of plant metabolism.

In the light of evidence now available, ammonia would be expected to react with precursors derived chiefly from carbohydrate metabolism; a cycle of transformations occurs in an organic acid series to provide these precursors. The suggestion is that from oxalacetic acid, $\begin{smallmatrix} COOH \\ CO \\ CH_2 \\ COOH \end{smallmatrix}$, asparagine is formed and from

THE CITRATE CYCLE OF KREBS
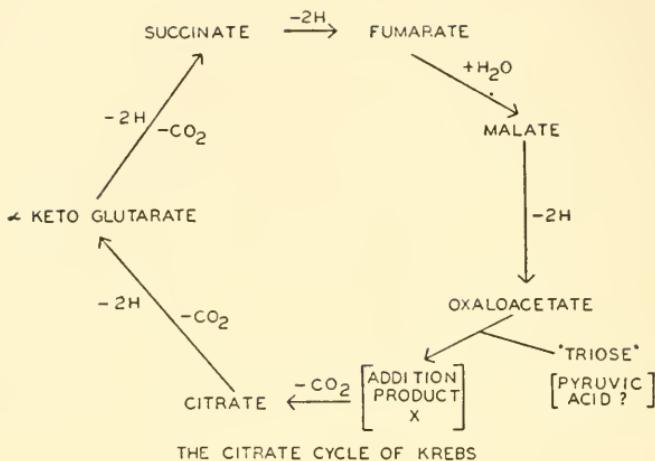
TEXTFIGURE 39. — One condensed represen-
tation of an organic acid cycle showing trans-
formations involving oxidation and reduction
reactions of possible interest in connection
with respiratory systems interrelated with salt
absorption. (From STARE and BAUMANN,
1939).

α-keto-glutaric acid, $\begin{matrix} COOH \\ CO \\ CH_2 \\ CH_2 \\ COOH \end{matrix}$, glutamine. In some plants

asparagine is the principal amide and in others glutamine. It is doubtful that most plants synthesize exclusively either asparagine or glutamine. A metabolic cycle that may explain the formation of either or both amides is one known as the citric acid cycle, which in one of its forms is illustrated by Fig. 39. Far more remains to be learned of the nature of the respiratory

$$
\begin{matrix}
COOH \\
CHNH_2 \\
CH_2 \\
COOH \\
\text{Aspartic} \\
\text{acid}
\end{matrix}
\quad
\begin{matrix}
+NH_3 \\
\longrightarrow \\
\longleftarrow \\
-NH_3
\end{matrix}
\quad
\begin{matrix}
COOH \\
CHNH_2 \\
CH_2 \\
CONH_2 \\
\text{Asparagine}
\end{matrix}
\quad +H_2O
$$

$$
\begin{matrix}
COOH \\
CHNH_2 \\
CH_2 \\
CH_2 \\
COOH \\
\text{Glutamic} \\
\text{acid}
\end{matrix}
\quad
\begin{matrix}
+NH_3 \\
\\
\longrightarrow \\
\longleftarrow \\
-NH_3
\end{matrix}
\quad
\begin{matrix}
COOH \\
CHNH_2 \\
CH_2 \\
CH_2 \\
CONH_2 \\
\text{Glutamine}
\end{matrix}
\quad +H_2O
$$
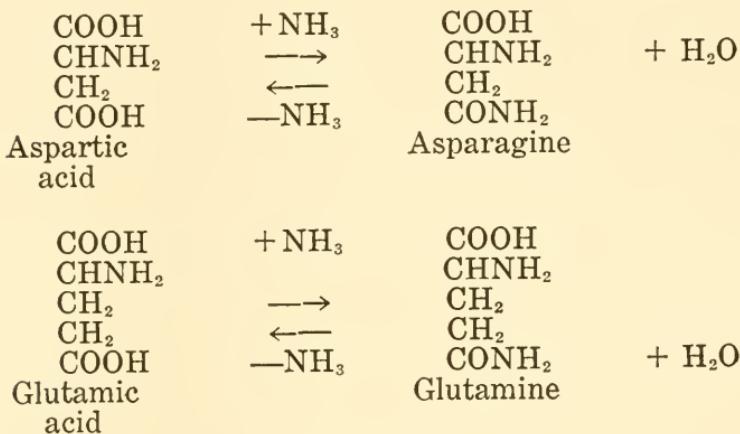
TEXTFIGURE 40. — Scheme of synthesis of asparagine and glutamine from amino acids.

processes in plants, and of the specific metabolic reactions that eventuate in amide synthesis. The degree to which the formation of the organic acid precursors may be stimulated by ammonia requires further elucidation. There are also to be explained the steps in synthesis that make possible the formation of the array of amino acids required for the building up of proteins.

The barley root tissues, as investigated in our experiments, do not accumulate appreciable amounts of ammonia, if they remain uninjured. Absorption of ammonia results in the formation of amides, primarily of glutamine. This amide can accumulate in the roots or be transported upward and appear in exudates.

During the absorption of nitrogen compounds, or as a result of subsequent metabolism, a large increase in total respiration has been found to occur in the excised roots of barley high in carbohydrate. In some experiments ammonia nitrogen seems to stimulate the rate of respiration to a greater extent than nitrate, although the latter also has a large effect. This respiratory response to absorbed nitrogen compounds has as a consequence an accelerated utilization of carbohydrate. In addition, carbohydrate content is reduced by the building up of soluble organic compounds of nitrogen, as has just been explained. Considering the intact plant, the net physiological result will depend on reactions of this type in various parts of the plant and on the photosynthetic activity of the plant as it is related to climatic complexes.

Metabolism and Accumulation of Ions: — A current study by MACHLIS of this laboratory has been devoted in part to testing the hypothesis that an organic acid cycle in a respiratory system may be linked in a chain of processes with ion accumulation by roots. The technique consists of making experiments on young barley roots by micro-respirometer methods and by measurements of ion accumulation through the use of radioactive bromide. It was noted in a previous lecture that a strong inhibitor of ion accumulation is iodoacetate, a respiratory inhibitor. The addition of suitable amounts of organic acids of the cycle to which reference has already been made overcomes this inhibiting effect on salt accumulation. Malonic acid

acts as an inhibitor in the organic acid respiration chain presumably because its constitution permits it to compete for sensitive groups in a protein enzyme, but without power to act as a hydrogen acceptor or donator. Malonic acid was observed to have an inhibiting effect on bromide accumulation which could be neutralized, at least partially, by supplying sufficiently large concentrations of one of the organic acids of the respiration cycle. Another type of respiratory inhibitor which can prevent ion accumulation is cyanide. It may be hoped that systematic studies on inhibitors will throw some light on the nature of respiratory systems that must operate to maintain the kind of cell metabolism essential to ion accumulation.

While the postulation may be made of the operation of one of the organic acid cycles, from indirect evidence, analysis of the roots discloses that malic acid forms by far the larger part of the acid present. A trace of oxalic and a small percentage of citric acid are also found. Most of the total acid is thus accounted for, by ISAACS, sometimes well over 90%, but this fact would not preclude the presence of other organic acids, formed in a cyclical process, effective catalytically, but too small in amount to give a significant value by the analytical methods utilized.

Ion Accumulation and Protein Metabolism: — STEWARD and his collaborators have repeatedly stressed, especially on the basis of experiments on potato storage tissues, that the accumulation of salt is dependent on metabolic processes intimately associated with cell growth and protein synthesis. During the process of salt absorption, when this brings about an increase in content of both cations and anions in the tissue, as occurs when potassium and bromide ions are actively accumulated, certain soluble nitrogen compounds are utilized in the building up of proteins. The view is held that this protein metabolism, if it does not have a direct part in the mechanism of salt ac-

cumulation, at least reflects a vital activity of living cells indispensable to the accumulation of salt in the sense indicated above. Protein synthesis is evidently not essential to certain types of salt accumulation, since STEWARD and PRESTON showed that from solutions containing bicarbonate, at relatively high pH, potassium could be accumulated, with the formation of organic acid, although evidence for protein synthesis was lacking. Accumulation of bromide, however, was suppressed. Possibly interrelations of bromide and bicarbonate ions in the absorption process were in part responsible for the latter effect.

In the studies of STEWARD and PRESTON on aerated discs of potato tuber accumulating both cations and anions of the salt presented (KBr), protein synthesis took place at the expense principally of amino-acid nitrogen other than that of asparagine or glutamine, although an unstable glutamine-like compound may be a reactive intermediary between stable reserves of amino acid nitrogen and proteins. An important conclusion of these experiments is that in the potato tuber tissue low respiration is associated with low protein synthesis and highest protein synthesis with highest respiration. Thus there are linked together protein synthesis, respiration, and salt accumulation. Potassium and calcium salts had opposed effects on the synthesis of protein and on respiration. The former accelerated, and the latter retarded these processes, at .075 molar concentrations. The full explanation awaits further investigation, but it may be of interest that some anions are absorbed more rapidly than calcium ions and effects of unequal ion absorption complicate the study of metabolic responses to calcium salts.

The excised low-salt, high-sugar, young roots of barley plants, which possess for a time an extremely high capacity for accumulation of both cations and anions from solutions of certain potassium salts, do not yield obvious evidence that growth processes and

protein synthesis are immediately concerned with salt absorption, although, as STEWARD has pointed out, the salt accumulating capacity of the roots is the result of active growth and protein synthesis in a preceding growth period, during which the roots could not satisfy their capacity to accumulate salt for the reason that the supply of the latter was deficient. Furthermore, in accordance with recent views based on isotope studies it seems conceivable that in the actively metabolizing roots transformations of proteins proceed rapidly even when no net changes in nitrogen components are demonstrable.

EFFECT OF SALTS AND AERATION ON THE NITROGEN FRACTIONS OF POTATO DISCS. ALL QUANTITIES OF NITROGEN IN MG. PER GRAM OF INITIAL FRESH TISSUE

Experimental treatment of discs	Protein N	Sol. N	Amino N			Amide N			Ammonia		
			Hot ext.	Cold ext.	Apparent "on-stable"	Hot ext.	Cold ext.	Easily hydrolyzable	Hot ext.	Cold ext.	NH₃ = to un stable amide
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
52.5 hours in aerated 0.00075 M KCl at 23° C.	0.92	1.15	0.90	1.04	0.14	0.129	0.305	0.176	0.178	0.002	0.176
53.0 hours in aerated 0.075 M KCl at 23° C.	1.21	0.65	0.57	0.676	0.105	0.156	0.270	0.114	0.120	0.002	0.118
Original washed discs	0.68	1.38	1.09			0.208	0.348	0.140	0.144	0.002	0.142

TABLE 5. — Effects of potassium salt accumulation and aeration on nitrogen metabolism of potato discs. (From STEWARD and PRESTON, 1940).

In the researches of THIMANN and his co-workers on coleoptiles, an inter-relationship was discovered among growth, protoplasmic streaming, sugar, auxin and catalytic organic acids. Some of these are the same factors concerned with the metabolic processes leading to salt accumulation by roots but we do not know how far we are justified in translating these results to the activities of roots in absorbing salt, especially since the effects of auxin on the growth of roots and on the coleoptile are not alike.

Progress in understanding the metabolism of plant cells in its relation to salt accumulation is limited by

deficiency of knowledge of respiratory systems in higher plants. Various systems apparently operate and the problem of their relative importance and roles in different processes remains to be solved. In the potato tuber tissue, as investigated by STEWARD and PRESTON, the accumulation of potassium salts is accompanied by oxidase activity, with the oxidation of phenolic compounds. BROWN and GODDARD find evidence that wheat embryos and barley seedlings contain a cytochrome oxidase, but in mature leaves no evidence for the function of this oxidase was found. The period has apparently arrived for more extensive exploration of respiratory systems of higher plants, comparable with that carried out by those who have worked on muscle and yeast.

At the heart of the whole question of salt accumulation in its relation to biochemistry is the nature of the energetic coupling of metabolism to the active transport of salt. At the present time no technique is available to measure the energy utilized in these particular processes. In theory, the expenditure of energy required would be extremely small. Speculatively, one possible approach to the problem might be envisaged as a development of a recently discussed theory of biochemists who have devoted themselves to research on biochemical processes in which energy is applied to other cellular activities. The notable example is found in studies on muscle.

The essence of the biochemical theory referred to has been outlined by LIPMAN (1941) and by KALCKAR (1941). The utilization of energy made available by respiration is associated with the formation and breakdown of phosphorylated compounds. Certain types of these compounds contain energy-rich phosphate bonds, from which energy can be yielded to cellular processes. The relation of the energy-rich phosphate linkages to contractility of muscle tissue has been more definitely considered than one that might be postulated for the movement of solutes against activity gradients. Con-

siderable attention, however, has been devoted to the absorption of sugar by animal tissues as governed by processes of phosphorylation. The view could be entertained that phosphorylations and dephosphorylations might create the necessary positive gradients, but it has also been suggested that the energy of phosphate bonds may be employed in accelerating the transport.

No definite proposal has been made for an explanation for the active transport of salt, such as occurs in root cells, based on the phosphate-bond theory, but the intimate relation between respiration and respiratory inhibitors and the accumulation of salt stimulates questions along this general line of hypothesis. It may also be true that a connection exists between the respiratory cycles, the building up and breaking down of protein molecules, or protein complexes, and the reactions of ions with basic or acidic groups of proteins, in a dynamic sense. However speculative such ideas may now be, it appears that progress in understanding the mechanism of salt accumulation will depend largely on advancing knowledge of the biochemistry of respiration, together with a correlation of biochemical transformations with the maintenance of organized structures in the protoplasm.

REFERENCES:-

ARNON, D. I. Ammonium and nitrate nitrogen nutrition of barley at different seasons in relation to hydrogen-ion concentration, manganese, copper and oxygen supply. Soil Science 44: 91-121, 1937.

—— ——. Effect of ammonium and nitrate nitrogen on the mineral composition and sap characters of barley. Soil Science 48: 295-307, 1939.

BROWN, ALLAN H. and GODDARD, DAVID R. Cytochrome oxidase in wheat embryos. American Journal of Botany 28: 319-324, 1941.

CHIBNALL, ALBERT CHARLES. Protein metabolism in the plant. Yale University Press, New Haven, Conn., 1939.

CLARK, HAROLD E. Effect of ammonium and nitrate nitrogen on the composition of the tomato plant. Plant Physiology 11: 5-24, 1936.

COMMONER, BARRY and THIMANN, KENNETH V. On the relation between growth and respiration in the *Avena* coleoptile. Journal of General Physiology 24: 279-296, 1941.

DUNNE, T. C. Plant buffer systems in relation to the absorption of bases by plants. Hilgardia 7: 207-234, 1932.

GREGORY, F. G. Mineral nutrition of plants. Annual Review of Biochemistry VI: 557-558, 1937.

HOAGLAND, D. R. and BROYER, T. C. Hydrogen-ion effects and the accumulation of salt by barley roots as influenced by metabolism. American Journal of Botany 27: 173-185, 1940.

KALCKAR, H. M. The nature of energetic coupling in biological synthesis. Chemical Review 28: 71-178, 1941.

LIPMAN, FRITZ. Metabolic generation and utilization of phosphate bond energy. Advances in Enzymology 1: 99-162, 1941.

LUNDEGÅRDH, H. und BURSTRÖM, H. Atmung und Ionenaufnahme. Planta, Archiv für wissenschaftliche Botanik 18: 683-699, 1933.

NIGHTINGALE, G. T. Nitrate and carbohydrate reserves in relation to nitrogen nutrition of pineapple. Botanical Gazette 103: 409-456, 1942.

PUCHER, GEORGE W. and VICKERY, HUBERT BRADFORD. Succinic acid as a metabolite in plant tissues. Plant Physiology 16: 771-783, 1942.

STARE, FREDERICK J. and BAUMANN, CARL A. Fumarates in biological oxidation. Cold Spring Harbor Symposia 7: 227-247, 1939.

STEWARD, F. C. Mineral nutrition of plants. Annual Review Biochemistry IV: 520-544, 1935.

—— ——. Salt accumulation by plants—the role of growth and metabolism. Trans. of Faraday Society XXXIII: 1006-1016, 1937.

—— —— and PRESTON, G. Metabolic processes of potato discs under conditions conducive to salt accumulation. Plant Physiology 15: 23-61, 1940.

—— —— —— ——. The effects of salt concentration upon the metabolism of potato discs and the contrasted effect of potassium and calcium salts which have a common ion. Plant Physiology 16: 85-116, 1941.

SWEENEY, BEATRICE MARCY and THIMANN, KENNETH V. The effect of auxins on protoplasmic streaming, II. Jour. Gen. Physiol. 21: 439-461, 1938.

ULRICH, ALBERT. Measurement of the respiratory quotient of plant tissues in a constant gaseous environment. Plant Physiology 15: 527-536, 1940.

—— ——. Metabolism of non-volatile organic acids in excised barley roots as related to cation-anion balance during salt accumulation. American Journal of Botany 28: 526-637, 1941.

—— ——. Metabolism of organic acids in excised barley roots as influenced by temperature, oxygen tension and salt concentration. American Journal of Botany 29: 220-227, 1942.

VICKERY, HUBERT BRADFORD and PUCHER, GEORGE W. The meta-
bolism of amides in green plants, III. The mechanism of
amide synthesis. Journal of Biological Chemistry 128: 703-
713, 1939.

—— ——, —— ——, LEAVENWORTH, CHARLES S. and WAKEMAN,
ALFRED J. Metabolism of amides in green plants, II. The
amides of the rhubarb leaf. Journal of Biological Chem-
istry 125: 527-538, 1938.

—— ——, —— ——, —— ——, —— ——. Chemical investi-
gations of the tobacco plant, VIII. The effect upon the
composition of the tobacco plant of the form in which nitro-
gen is supplied. Connecticut Agr. Exp. Sta. Bull 422, 1940.

ASPECTS OF THE POTASSIUM NUTRITION OF PLANTS AS ILLUSTRATING PROBLEMS OF THE SYSTEM, SOIL-PLANT-ATMOSPHERE

INTRODUCTORY STATEMENT

About fifteen years ago several members of the Agricultural Experiment Station in California undertook an investigation of a disease of prune trees occurring in the upper part of the Sacramento Valley, which presented a problem of practical importance. The trees in the affected districts often grew for five to eight years in a satisfactory way and then, when they came into heavy bearing, the so-called "die-back" disease appeared, although at least one type of soil produced injury at an early stage of growth. One of the chief symptoms of injury was a scorching of the leaves, occasionally preceded by a slight chlorosis. The scorching of leaves assumes various patterns and sometimes is accompanied by the dying of small circular areas of tissues, which fall out and leave a "shot-hole" effect. After a period of severe injury the upper part of the tree dies back. In the course of several seasons severely injured trees may die or become worthless.

While some aspects of the injury to the trees suggested potassium deficiency as one cause of the trouble it was at first deemed doubtful that this factor was concerned, since potassium deficiencies in California soils were not expected, especially in the growth of fruit trees. By general inspection the soils in which

the prune trees were planted were rated as of excellent character agriculturally. Nevertheless, preliminary study of the soils led to the belief that they might be lacking in power to supply potassium to the trees at an adequate rate. The first chemical examinations of samples of soil from some badly affected areas showed in particular very low concentrations of soil solution potassium and also low amounts of potassium that could be released in the base exchange process, which has been given some attention in an earlier lecture. These data were not in themselves conclusive of a potassium deficiency, as we shall later show, but they gave reason for an investigation of this possibility. (*See plates 27 and 28*).

In the subsequent years, the investigation expanded into a diversified study of the potassium nutrition of plants in relation to soil problems, which embraced laboratory, greenhouse, and field experimentation*. It happened also that in this period there was a recrudescence of interest by various investigators in the status of soil potassium, in part based on the development of knowledge of the crystal structure of soil colloids. The results of general research on potassium now available present, therefore, an opportunity to illustrate the nature of an attack on a problem of the system, soil-plant-atmosphere. The immensity of the literature dealing with potassium and plant growth of necessity limits this discussion to several of the broader aspects of the subject.

The thought might occur at first to many persons that if a deficiency of potassium were an important

*For many years Dr. O. LILLELAND has conducted field experiments with trees, accompanied by laboratory studies, and various other members of the Experiment Station have made researches on the general aspects of potassium nutrition of plants. In one experiment, large lots of soil were transported to Berkeley and placed in cylinders holding 1000 pounds of soil. Prune trees were grown under these conditions for about seven years and chemical examinations were made on the soils and tissues of the trees.

factor in the prune die-back disease, then a simple cure would be found in the application to the soil of potash fertilizers. Actually several instructive difficulties are met in the effort to overcome the conditions of disease in the trees by adding fertilizers to the soil or by other soil treatments. These difficulties involve both the physiology of the plant and the chemistry of the soil. First I shall discuss several features of soil systems in relation to the potassium status of soils.

Reactions of Potassium in the Soil: — In a previous lecture, stress was given to the point that when a fertilizer salt is applied to soil this is not merely a process of adding something to an inert medium. Potassium can react with soil colloids by a base exchange reaction, displacing calcium, magnesium or sodium ions. By this reaction, potassium becomes "fixed" by the colloid. In turn it can be displaced by other cations, including hydrogen ions. The exchangeable potassium later becomes available to plants through this exchange, since hydrogen ions are produced by root respiration or the metabolic activities of microorganisms. The equilibria of the system are constantly disturbed because of the rapid absorption of potassium ions by roots. In order that this potassium may become effectively available to plants, the roots must act close to or in contact with colloidal particles on which the potassium is held.

If most of the absorbing roots are active below the zone of fixation, but little of the added potassium may reach them within the necessary periods of time. The roots of fruit trees, under some agricultural conditions, do not develop absorbing systems in the upper zones of soil in which the fixation of potassium first occurs. If surface applications of fertilizer are made, a problem of fertilizer placement arises in those soils that have a high fixing power for potassium, when deep rooted plants must be treated.

At first, in the researches in California on the potassium problem chief attention was given to the fixation of potassium in the replaceable form. At the time it was supposed that at least the rapid fixation of potassium was primarily in this form. But soon it became clear that some of the potassium added to certain soils could also undergo fixation of such a nature that the potassium could not be easily replaced by another cation. This fixation may take place quickly and thus not require long-time weathering processes in the field. The potassium entering this form has been termed "non-replaceable," on the basis of criteria provided by chemical procedures commonly used in the laboratory for estimating replaceable potassium. It is not to be inferred that a sharp line of demarcation can be drawn between replaceable and non-replaceable potassium, but a valuable differentiation of degree of resistance to chemical reagents can be made.

Many studies have been conducted in recent years by various investigators on fixation of potassium by soil colloids in the non-replaceable, or difficultly replaceable form, but not all the questions have been satisfactorily answered. The fixation of potassium by this mechanism is generally increased by wetting and then drying the soil. Sometimes repetition of the wetting and drying treatments augments the fixation in non-replaceable form. One tentative explanation of this phenomenon given by several investigators rests on a property of certain types of soil colloids that have lattice structures capable of expansion and contraction under different degrees of hydration, as is true of colloids of the montmorillonite type (see illustrations in Lecture 1). According to this hypothesis potassium ions would react with the colloid and the lattice would contract, especially when drying occurred, preventing the subsequent replacement of the potassium ions, although according to our experiments the bases calcium, magnesium and sodium are first released in amount almost equivalent to the total

amounts of potassium fixed, in *both* replaceable and non-replaceable forms. The explanation advanced above would imply a reaction with potassium that would decrease the exchange capacity of the colloid, by an amount equivalent to the potassium fixed in non-replaceable form.

This decrease in exchange capacity is realized in experiments on bentonitic colloids, and on some colloids separated from soils, by ascertaining the exchange capacity of the colloids by means of determinations of the total amounts of ammonium ions capable of adsorption by the colloidal preparations, before and after the fixation of potassium in non-replaceable form. Demonstration of this loss of exchange capacity is not always so easily performed on the entire soil. There are in fact many unsolved problems of potassium fixation, which belong to a special field of study in soil chemistry. From the point of view of plant nutrition we are more immediately concerned with the relations of the various forms of potassium combinations to the capacity of the soil medium to supply potassium to plants at a rate commensurate with their physiological requirements.

The Capacity of the Soil to Supply Potassium to Plants: — What is the relation of the solution to the potentiality of the soil to supply potassium to plants? An earlier lecture presented the point that not often is the total amount of potassium present in the soil solution at any one instant, even in the whole mass of soil accessible to the roots, sufficient to meet the needs of the plant over its entire growth period. On the basis of the soil solution theory of absorption of nutrients by plants, potassium would have to enter this solution from the solid phase of the soil as absorption by the plant takes place, rapidly enough to prevent the concentration of potassium from falling to a critically low value at any time, in relation to physiological requirements of plants at various stages

of crop growth; granted that these requirements would not necessarily imply the maintenance of soil solution concentrations as high as those found at the beginning of crop growth.

We know from nutrient solution experiments of the kind already described that the potassium required by plants can be furnished from solutions in which the potassium level does not exceed a few parts per
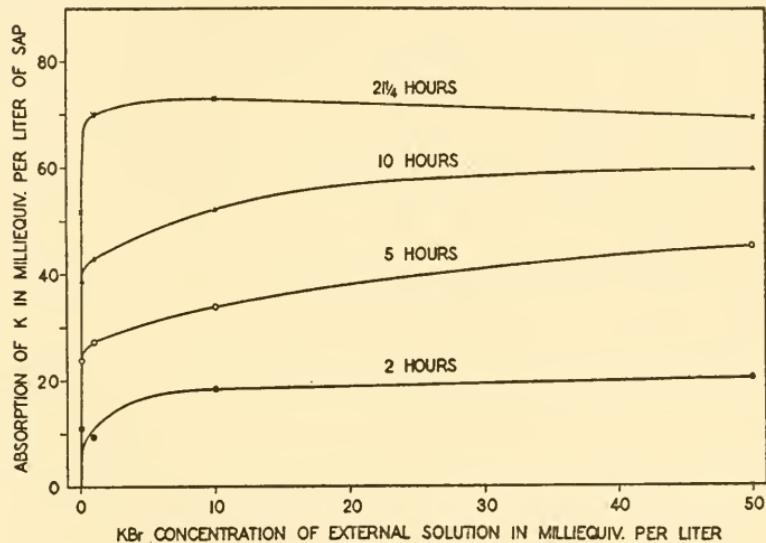
TEXTFIGURE 41. — Absorption of K by excised roots of young barley plants as influenced by concentration of potassium in the external solution. (From HOAGLAND and BROYER).

million of solution, provided the concentration is properly maintained by flowing solutions, or by making accessible to plant roots large volumes of stirred solution. From a dilute nutrient solution plants can absorb more potassium relative to concentration than from one of higher concentration. Experiments with tomato plants have indicated that a flowing solution containing not more than five parts per million of potassium suffices for good plant growth, at least in the vegeta-

tive stages. Probably even lower values would be adequate if present methods of aerating solutions were employed.

The adaptation of plants to extremely dilute potassium solutions is achieved because of the remarkable ability of roots to absorb potassium ions rapidly when the roots are growing and accumulating potassium by metabolic processes. Despite this important consideration, soil solutions from some soils are found in which concentrations of potassium are maintained at such low levels that doubt arises that the concentrations are really adequate, or above critical levels. Nevertheless, certain of these soils supply enough potassium to plants as evidenced by the growth and yield of the latter. It is difficult, however, to evaluate the efficacy of the soil solution concentrations as ascertained because of uncertainty as to the total extent of absorbing root surfaces. Furthermore, we do not have accurate knowledge of soil solution concentrations in localized zones of the soil or of rates of entry of potassium into the soil solution.

If the soil solution seems to fail as an intermediary for the transfer of potassium from the solid phase of the soil to the plant root the direct contact theory of absorption outlined before, may be invoked. Complete proof that this method for the absorption of potassium must perforce operate to explain the results of the experiments on plants growing in soils, is not yet available, but I have mentioned elsewhere that JENNY, OVERSTREET and others have offered several lines of evidence that contact absorption of some ions by roots does take place.

However this question of mechanism may be decided, it is clear now that when a root grows in contact with or close proximity to colloidal particles bearing potassium ions in replaceable form, these ions are in general readily available to the plant. Hydrogen ions act as chief replacing agents. Thus a soil with a relatively large amount of replaceable potassium is not

likely to be deficient in potassium supplying power. The amount of replaceable potassium need not be high in an absolute sense—even a few hundred parts per million, calculated on the basis of dry soil, may be considered as high from this point of view.

The availability of replaceable potassium to plants is a function of the degree of saturation of the colloid with potassium (the proportion of potassium ions to the total number of adsorbed ions), as well as of the total amount of replaceable potassium. Availability further depends on the kinds of other ions adsorbed by the colloid along with potassium, as JENNY and AYERS (1939) have shown. If hydrogen ions are the displacing ions, then they may be used either in displacing potassium or other adsorbed ions and the relative strength with which ions of different kinds are held by the colloids is of importance. Adsorbed calcium, for example, is held more tightly by the clay particles than sodium is. Consequently hydrogen ions displace potassium more readily from colloids containing calcium as a complementary ion than from those containing sodium. The kind of colloid on which the ions are adsorbed is likewise of consequence.

Not less significant than these physical-chemical factors is the biological one; namely, the rapid and selective removal of potassium by the action of plant roots. (The enormous absorbing area developed by roots should once more be recalled). This is a point that justifies the repeated emphasis I have given it. The action of plants can cause the replaceable potassium to be reduced to a very low value in an absolute sense, and also in proportion of potassium to total adsorbed bases. At least this is true of many systems in which calcium predominates.

In these systems a relatively high content of replaceable potassium in the soil is reflected in a relatively high concentration of potassium in the soil solution. With a given soil, the addition of potassium salts in successive increments results in successively

increasing concentrations of potassium in the soil solution, even though most of the added potassium is fixed by the soil colloids. As the potassium increments are made larger, the percentage fixation of potassium diminishes, as would be expected. Whether the plant utilizes replaceable potassium directly (contact effect) or through the medium of the soil solution, cropping tends to reduce the concentration of potassium in the latter. While, as already explained, plants absorb more potassium proportionately to concentration from solutions of decreasing concentration, within certain limits the absolute amounts absorbed per unit of plant tissue increase with increasing soil solution concentrations (and increasing values for replaceable potassium) and so the percentage content of potassium in the plant rises to a maximum value. This value depends on physiological and genetic factors.

These explanations do not cover all the pertinent phenomena. There are soils that supply enough potassium for the production of excellent crops, which nevertheless contain only very small amounts of replaceable potassium at any time, amounts which do not decrease with continued cropping; and at the same time the soil solution concentrations are low. We then say that the crop is, in effect, deriving potassium from non-replaceable, or very difficultly replaceable, form. The evidence for this statement is extensive and includes results obtained by this laboratory in a greenhouse experiment in which numerous soils were successfully cropped over a period of years, with quantitative determinations of the potassium withdrawn from the soils by the plants and chemical studies on the soils themselves.

The conclusions have a practical bearing on attempts to ascertain the "available" potassium in soils by simple chemical examinations, as by using dilute acids to extract potassium. The point will perhaps be made clearer by citing an experiment by MARTIN

and OVERSTREET* in this laboratory.   In this experiment potassium behavior was reflected by the element rubidium, which can be obtained in suitable long-lived radioactive form.   There is every reason to believe that for the purpose in view rubidium presents a

O   WATER   SOLUBLE   K   AGAINST   ADDITIONS   K   TO   SOILS A AND B
●   WATER   SOLUBLE   K   AGAINST   REPLACEABLE   K   IN   SOILS A AND B
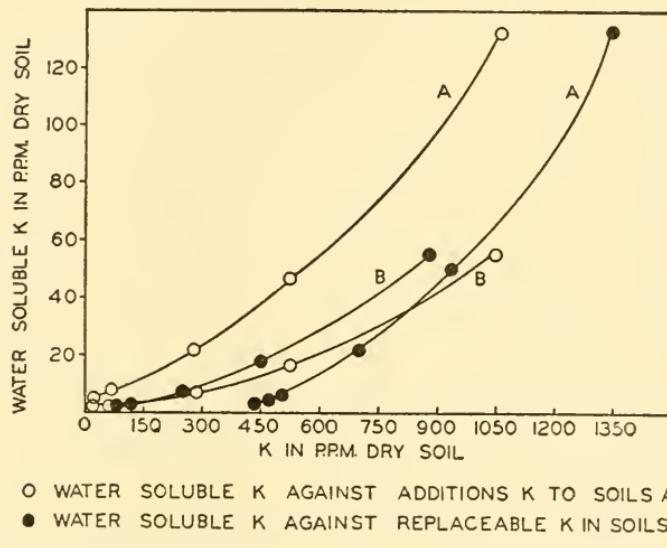
TEXTFIGURE 42. — Showing increases in soil solution K (as reflected in water soluble K) with increasing additions of K salt to the soil and concomitant increases in replaceable K. The larger part of the added K may be fixed on solid phases of the soil, in soils of this character, and not appear in the soil solution.   (HOAGLAND and MARTIN, 1933).

behavior analogous to that of potassium.   Radioactive rubidium was added to the soil under study and then the soil was leached with a calcium salt so as to remove all the rubidium present in easily replaceable form, leaving a considerable portion of the rubidium, which had been fixed in non-replaceable, or difficultly replaceable, form.   Subsequently barley plants were grown in the leached soil.   They absorbed significant amounts of rubidium.   Since the rubidium was tagged

*Private communication.

by its radioactivity there was no doubt that the plants had absorbed the particular ions which had been added and fixed in a chemically or physically more or less resistant form.

Many other experiments furnish evidence of the absorption by plants from some soils of potassium that is difficult to remove by laboratory procedures with reagents that might be thought to have effects of biological significance. An example from the experience of this laboratory consisted in a comparison of the amount of potassium leached from a soil by ten days continuous leaching with carbonic acid saturated water, with the amount of potassium absorbed in a similar period by actively growing rye seedlings. The plants withdrew from this soil more than twice the amount of potassium leached from it in the laboratory by the carbonic acid.

A suggestion advanced by one investigator is that soils that supply much potassium to plants from non-replaceable form are generally rich in certain forms of micaceous minerals. The assumption can be made that the total amount of the effective minerals present in the soil and their state of subdivision are important in determining the number of points of root contacts.

The direct contact theory of absorption of ions may be of value for attempts to understand these phenomena. While studies on the contact mechanism have dealt experimentally with readily replaceable cations, the whole system of soil solution potassium, replaceable potassium and non-replaceable potassium, tends toward an equilibrium state, which, however, is not attained because of biological interference. Non-replaceable potassium ions may migrate into replaceable positions, as ions are removed by plant action either from the soil solution or directly from colloids by contact effects. Gradual hydrolytic breakdown of resistant potassium minerals may also supply potassium ions to the soil solution and these would be available for reactions with the colloidal system.

We need to understand the chemistry of the soil as it pertains to potassium, but the immediate way
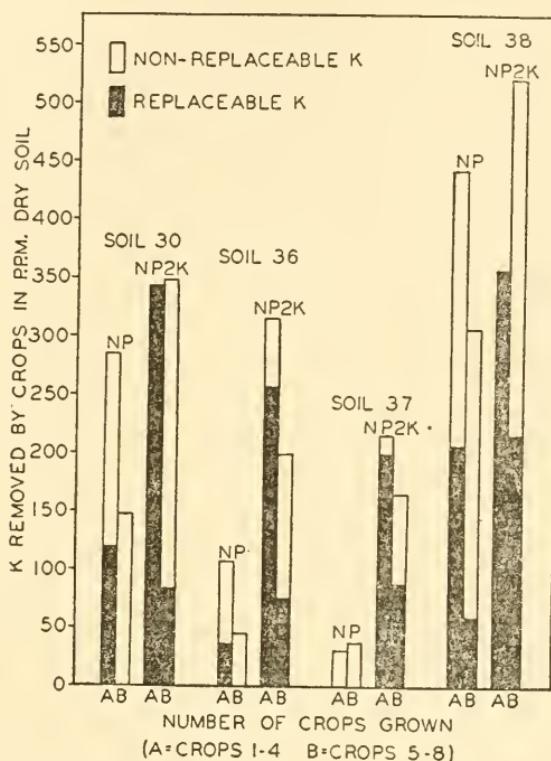
TEXTFIGURE 43. — The absorption of potassium by crops from replaceable and non-replaceable forms, as influenced by characteristics of soil and duration of cropping. Increasing amounts of potassium, referable to the non-replaceable fraction, are absorbed as cropping reduces relative amounts of replaceable potassium. (HOAGLAND and MARTIN, 1933).

to find out how much potassium is in a condition to be absorbed by plants is to determine how much potassium plants do absorb over long or short periods of time under conditions which are not otherwise

limiting for plant growth. There is difficulty in ascribing the potassium absorbed by plants to some particular form of combination in the soil if the whole system is in dynamic equilibrium.

Since the total amount of potassium in a soil is sufficient for the requirements of plants for many years, if potassium in all the forms present could be yielded to plants at an adequate rate of supply, there would not be found any soil in which crops would suffer from a potassium deficiency, which is obviously contrary to fact. In many soils the potassium is not capable of release to plants at rates commensurate with their requirements. Consequently it is necessary to distinguish different degrees of absorbability by plants of non-replaceable potassium. Potassium initially present in the soil can be chemically too inert to insure adequate absorption by plants, and some of the potassium added in fertilization may also become so fixed that it is not only non-replaceable by chemical tests, but also more or less non-available to plants.*

At present it does not appear that the physiological efficacy of the potassium in soils can be consistently appraised without the aid of experiments with plants, of one kind or another, however useful some workers may find certain soil examinations, when these are associated with much experience with particular crops and types of soil. On a laboratory scale the biological test known as the Neubauer method, in which a large number of rye seedlings are grown in a small amount of soil mixed with sand, has been helpful in certain types of study. There is also some promise in the analysis for potassium of a suitable portion of the

*The interesting observation has been made in our experiments, and in those of one or two other laboratories, that continued cropping of some soils leads to a fixation of part of the potassium initially added to the soil, in a difficultly replaceable and root absorbable form. This effect may occur even in soils that show very little fixation of potassium in resistant form under laboratory conditions.

plant at appropriate stages of growth, as the crop is growing in the soil it is desired to appraise.

Using procedures like these implies adoption of the view that the plant itself is the best indicator of what it can absorb. But whatever sort of examination is made on a small scale, there is always to be faced in its application to practical agriculture an array of factors influencing the growth of crops and many of these factors can be only crudely predictable or are too little understood to be assessed. Thus the improvement of crop production by fertilizer applications of potassium or other elements rests on combining and interpreting knowledge and experience of many kinds.

A Physiological Aspect of Potassium Supplying Power of Soils: — In appraising the requirements of a crop for potassium there is one physiological factor that demands special attention, and here I should like to return to the prune die-back disease. This disease appeared, except in the most extreme type of potassium deficient soil, as a sequence of heavy bearing of fruit. In the localities concerned the trees tend to set far more fruit than similar varieties of trees do in most other localities. This tendency to heavy bearing is seemingly the result of an interreaction of climatic factors, not yet clearly understood.

The development of a heavy prune crop means a great draft on potassium, which migrates from the foliage into the fruit. The too great depletion of potassium from the tree, or metabolic disturbances resulting from this depletion, can be assigned as a cause of injury. In other words, the capacity of the tree to absorb potassium is not great enough to meet the physiological requirements of the entire tree, including the developing fruit. LILLELAND and BROWN (1938) have shown that removing the fruit at an early stage prevents the appearance of dieback symptoms, assuming the soil condition is not too bad. It

is true that phosphate and other nutrients are also mobilized in the fruit and that large amounts of sugar or other organic compounds are transported in this direction. The symptoms of injury to the plant suggest, however, even though they may not conclusively prove, that potassium is the critical determinant.

Somewhat analogous results have been obtained with heavily fruiting tomato plants grown in a greenhouse in Berkeley. Here, in aerated culture solutions it is possible to prevent almost entirely symptoms of potassium deficiency, despite very heavy fruiting, by maintaining suitably high concentrations of potassium in the well aerated nutrient solution. Frequently the prune trees have not been maintained in health, even when so much potassium was added to the soil, at suitable depths, that the foliage content of potassium early in the summer was raised to a high percentage.

The development of a heavy crop reduces markedly the potassium content of the foliage early in the fall. Whether the relation is direct or indirect, the excessive migration of potassium is accompanied by dieback injury. I have been speaking of extreme cases. There are conditions under which leaf scorch and similar injury to fruit trees can be corrected by practical applications of potash fertilizers to the soil.

Interrelations of Bases in Absorption and the Role of Potassium in Plant Sap Buffer Systems: — The absorption of potassium by roots is related to the absorption of other ions as well as to the chemical nature of potassium compounds in the soil. This is another physiological aspect of the problem. Of particular interest is the interrelationship in absorption of potassium, calcium and magnesium. Manifold researches have shown that the alteration of a soil or of a culture solution medium so as to promote the absorption of potassium will in general tend to decrease the absorption of calcium and magnesium, especially on the basis of the amounts of these elements absorbed per unit

of plant tissue produced. The percentage content of a plant in calcium or magnesium, or both, will decrease concomitantly with increase of potassium. Conversely, an increase in the supply of absorbable calcium or magnesium may tend to decrease the absorption of potassium from a low potassium medium, although
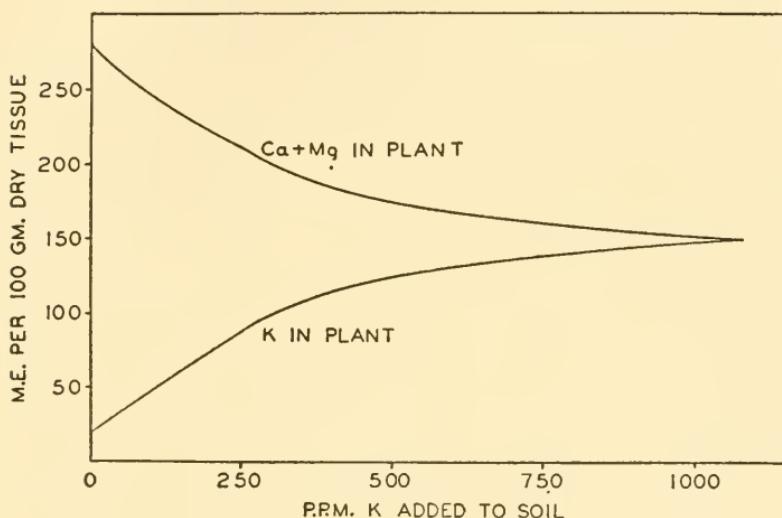
TEXTFIGURE 44. — With increasing additions of K salt to this soil the calcium plus magnesium absorbed by tomato plants decreased (per unit of tissue). Many chemical reactions occur in the soil, but the effects on absorption of K and of Ca and Mg are considered as primarily related to physiological interrelations in ion absorption. Similar effects can be obtained in nutrient culture solutions. (HOAGLAND and MARTIN, 1933).

this effect is likely to be far less marked than the effect of potassium on the absorption of calcium and magnesium.* The presence of ammonium ions in the medium has the tendency to retard potassium absorption be-

———.
*Within certain ranges of dilute solutions, calcium may accelerate the absorption of potassium ions and the absorption of anions as well, in short time studies on metabolically active excised young barley roots, according to VIETS in this laboratory. Another type of physiological effect than the one discussed above is probably in operation under these special conditions.

cause of interionic competition. The absence of nitrate is also to be considered since the absorption of potassium tends to be accelerated by the absorption of a mobile anion like nitrate.

The quantitative relations among basic ions absorbed: namely, potassium, calcium, magnesium and sodium are of considerable physiological importance. It is not unusual to find that the decrease in absorption of one base will be compensated roughly by an increase in absorption of other bases, so that the total equivalents of bases present in the plant tissues will remain approximately constant. But this does not always occur. The decreased absorption of potassium will not necessarily be fully compensated by the increased absorption of other bases. Many types of plants absorb potassium much more readily than calcium or magnesium, although the impression should not be held that potassium ions are always more mobile in absorption than calcium, magnesium or sodium ions. The work of COLLANDER illustrates that the genetic characteristics of the plant as well as the physical-chemical characteristics of the ions determine the relative amounts of different ions absorbed from solutions containing equivalent concentrations of the several bases. Certain leguminous plants, for example, absorb calcium about as readily as potassium. So do some non-leguminous species.*

Leaving aside the cases in which calcium, in terms of equivalents, can gain entrance into the plant at a rate comparable with that for the entrance of potassium, a general survey of knowledge of plant nutrition leads to the conclusion that for most types of higher plants for which evidence is available, the building

*Often the question presents itself, of how the amounts of nutrients absorbed should be computed, whether as total amounts per unit number of plants, or per unit mass of soil, or as percentage of tissue produced by the plant. The former method is required for some purposes, but the latter is probably more enlightening in discussing the problems now under consideration.

up of a high potassium content is effected with special facility. We speak of a "luxury" absorption when the content of potassium in the tissues increases without increase of growth.

There is also another physiological aspect of the interrelations of bases after these have been absorbed by the plant. The major part of the potassium in plant tissues is located either in the cell sap or, if not, is at least easily soluble in water; although a small proportion of the potassium can exist in difficultly soluble form in some plants. On the other hand, a much larger percentage of the calcium and magnesium is found in difficultly soluble form. It has long been known that in some species of plants calcium oxalate is present and forms crystals or precipitates. Pectin or protein compounds are capable of combining with or adsorbing calcium or magnesium ions.

Some of these facts have a significance with reference to plant sap buffer systems that is not always appreciated. If the supply of potassium from the nutrient medium is low and the potassium content of the plant tissues is diminished as a result, the content of fixed base in the sap decreases if much of the calcium and magnesium goes out of solution in the plant, even though the content of total calcium and magnesium in the tissues as a whole increases by amounts equivalent to the decreased absorption of potassium. Many species of plants do not absorb sodium rapidly enough to substitute for potassium for this purpose, or sodium may not be available in sufficient amounts in the medium.

I should like to present a specific consideration from the studies on the potassium nutrition of prune trees to which I referred at the opening of this lecture. The observations to be described were made on trees grown in Berkeley in cylinders of soil of one thousand pounds capacity. While the potassium deficient trees usually contained in their leaves a higher percentage of total calcium and magnesium than was found in

the trees receiving an adequate supply of potassium, hardly any of the calcium was present in dissolved form and only part of the magnesium. In the particular circumstances sodium was not an important constituent of the tissues.

The concentration of potassium was very low in the expressed sap of the leaves of the potassium deficient trees and the deficit of potassium base was not compensated for in this system except in some measure by magnesium. This means that in the normal trees potassium and magnesium were the principal bases in the leaf sap, with potassium predominating, while in the potassium deficient trees the total content of fixed bases was low and the relation of potassium to magnesium was greatly altered (magnesium increasing in relation to potassium), with whatever influence these alterations in base relations have on the protoplasmic colloids in contact with the sap. Another result was that the pH of the expressed leaf sap of the deficient trees was significantly lower than that of the normal trees. As bearing on these relations, the organic acid system discussed in the preceding lecture should be kept in view. The effects I mention were apparent before the leaves reached a stage at which external signs of injury became evident but one can reasonably assume that changes in the chemical constitution of the leaf were the cause of the subsequently manifest injury.

The relations of potassium, sodium and rubidium in the physiology of the plant have received extensive discussion during the history of plant nutrition. Potassium and rubidium are closely similar in many chemical properties, so much so that their analytical separation is difficult. Yet rubidium cannot be substituted for potassium in the growth of higher plants and the former element may have a toxic effect not produced by potassium. For algae and fungi statements have occasionally been made that rubidium can take the place of potassium, but there is apparently no

conclusive proof of this. Any preparations of rubidium salts that have been used probably were not free of potassium. There is no evidence that sodium can replace potassium except in part.

A distinction should be made between partial and complete substitution of chemical elements. The latter is meant in connection with the remarks just made. Even though complete physiological replacement cannot be effected, it would still be possible that a degree of substitution could occur. There is some reason to believe that in this sense sodium can decrease the potassium requirements of plants. It is not difficult to understand that sodium might partially take the place of potassium as a component of the sap buffer system, but there is the limitation in this action, as already noted, that many species of plants do not absorb sodium from dilute solutions as rapidly as they absorb potassium.

These various arguments point to the importance of potassium in many plant buffer systems. At the same time it is necessary to recognize that some kinds of plants do ordinarily contain a large amount of calcium and magnesium in their sap and that these ions are likewise of importance in the plant buffer systems. The alfalfa plant might be mentioned as one that normally not only has a high total calcium content, but also a considerable amount of calcium in soluble form and presumably present in the cell sap.

Other Functions of Potassium: — The impression should, of course, be avoided that the function of potassium is restricted to a role in buffer systems. There must be other functions of this element and they are, unfortunately, still to a large extent obscure. In the general literature of plant science a favorite idea is that potassium is essential for photosynthesis or for carbohydrate metabolism. It is perhaps not inappropriate to remark at this point that since potassium is an essential element for plant growth it is

ultimately necessary for every kind of synthesis or metabolism in the plant. The question at issue is how direct a role does it play in any given physiological process.

One way to approach this problem is to study plants subjected to potassium deficiency for limited periods of time and observe some of the effects on organic constituents of the plant. I should like to cite for illustration a series of experiments in which I cooperated with Professor A. R. DAVIS. These experiments were carried out with young wheat plants grown in temperature controlled chambers under known artificial illumination. Some sets of plants were furnished nutrient solutions with a supply of potassium inadequate to produce the full growth of the plants that was possible under the given climatic environment, but not so inadequate as to produce a marked degree of potassium starvation. For analysis, the shoots of the plants were divided into upper and lower portions, also the roots.

The total sugar concentrations of the potassium deficient plants were decidedly greater than those of the plants receiving the larger supply of potassium. In one experiment at least, the total amount of sugar per unit number of plants was approximately the same for the low and high potassium plants, despite the smaller size of the deficient plants. It did not appear that over the experimental period the potassium deficient plants were retarded in their growth directly by lack of carbohydrate synthesis or translocation. Also, some evidence from these and similar experiments, and a large amount of evidence by other workers, indicates that soluble organic nitrogen is often higher in concentration in low potassium plants than in high potassium plants.

A suggestion is derived that potassium is essential for some step in protein synthesis. In line with this concept are the data by STEWARD and his collaborators on biochemical changes occurring during the absorp-

tion of potassium by potato tuber discs. Possibly this function of potassium is of primary importance in the synthesis of various kinds of proteins formed by meristematic cells. Certainly potassium accumulates rapidly at growing points, and readily migrates from older tissues to meristematic regions.

Conclusions about the possible direct or near-direct function of potassium in protein synthesis will vary depending on the kind of experiments from which conclusions are drawn. The stage of plant growth, the interactions of nutrients, and degrees of potassium deficiency all complicate the issue. Statistical correlation of factors may provide illumination, but the use of this tool cannot elucidate the actual mechanism by which potassium operates in the plant.

With regard to the condensation of sugar units to form starch or other polysaccharides there is lacking sufficient proof that potassium is immediately essential for these processes, while phosphate has been shown to be necessary for the *in vitro* synthesis of starch. In some experiments potassium deficient plants have been observed to be readily capable of starch formation or digestion.

Some of these views might seem to be contradicted by studies here and elsewhere in which barley plants have been grown throughout their cycle in potassium deficient soils. The result then is that the total carbohydrate content of the plant is decreased, and the grain is shrunken in comparison with that of plants receiving a full supply of potassium. There is no necessary inconsistency in the observations from the two types of experiments. When plants are grown for a long period under potassium deficiency, carbohydrate synthesis and metabolism will be affected, no matter what the essential function of potassium may be.

There is no disproof of the assumption that potassium may be more or less directly essential to the photosynthetic process. In our young low-potassium

wheat plants there may still have been enough potassium available to accomplish a function in photosynthesis, so that for this purpose potassium was not a limiting factor. Perhaps some of the most direct evidence that potassium may have a relatively immediate role in photosynthesis comes from experiments by PIRSON (1939) on *Chlorella* cells. These cells when in a low potassium status, responded quickly in increased photosynthesis to the application of potassium (also of rubidium). The results of other types of experiments on carbon assimilation performed on leaves of higher plants in recent years also have been interpreted as evidence of a direct function of potassium in this process. Even so the entire photosynthetic system is exceedingly complex and involves various types of metabolism.

In this problem, as in nearly all others of plant nutrition, we do not deal with single limiting factors. One factor interacts with another. The climatic environment influences the requirement of the plant for potassium, or its ability to absorb potassium. I recall an investigation made many years ago in our laboratory on the effects of potassium concentration in a nutrient solution on the growth of the tomato plant (JOHNSTON and HOAGLAND, 1929). Some plants were grown in solutions of deficient supplying power for potassium, some in solutions of high potassium supplying power. Under each potassium condition, part of the plants were grown in the full light of the greenhouse and part under the shade of a cheese-cloth covering. In a sense two limiting factors could operate at once. Considering the low potassium plants, plant growth could be increased in the shade by increasing the potassium supply and at the same level of potassium, yield could be increased by improving the light condition.* The Rothamsted experiments on barley

*Doubtless one effect may have been related to this indirect effect of light on root activities and their potassium absorption.

are often cited as evidence that potassium fertilization is more effective in the years of unfavorable weather. Some results from experiments conducted in the controlled chambers at Berkeley suggested an inter-reaction of light and potassium. But this view is not necessarily supported by all available evidence.

Relations of Nitrogen, Phosphorus and Potassium:— Much has been said about the balance of nitrogen, potassium and phosphate in the nutrition of plants or in the practical application of fertilizers to the soil. With regard to this question, one needs to remember, as one point, that a balance is not established in the soil in direct accordance with the balance in the fer-tilizer added. The chemical reactions of these three nutrients with soil components are not alike. Potas-sium and phosphate are fixed by soil colloids or other components of the soil and by different reactions. Nitrate is not fixed to an appreciable extent, although ammonium may be temporarily. A soil balance of nitrogen, phosphate and potassium, physiologically con-sidered, must be based on the status of the soil as affected by the chemical and biological reactions of soil and fertilizer. It has already been noted that potassium is fixed by soil components in several ways. Moreover, within the plant itself the balance that is determinative of plant growth is not confined to the proportional relationships of inorganic nutrients. The significant balance is rather governed by the inter-reactions of inorganic nutrients with the carbon com-pounds synthesized and metabolized. Potassium is one of the elements required by the plant system as a whole. It is self-evident that if nitrogen forms a limiting factor for growth, an increased supply of nitrogen will entail a greater demand for potassium and vice versa. But the reduction of nitrate and the metabolism of nitrogen compounds are also dependent on available carbohydrate, and on all those phenomena that are associated with organic catalysts, the enzy-

mes, and no doubt the correlating action of hormone substances.
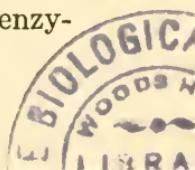
To determine by empirical means under a given climatic and soil condition, for a particular crop, at a particular stage of growth, that the growth of the plant, with a resultant high or low yield, is accompanied by a certain relation of percentages of nitrogen, phosphorus and potassium present in the tissues is one thing. To explain the physiological events in which these nutrients participate is another. Even for a practical purpose the utilization of nitrogen, phosphorus, potassium and other inorganic nutrients should be evaluated in relation to climatic and physiological factors influencing the organic metabolism of the plant. Assuming that a given climatic complex is operating, there are interrelations among nutrient elements that may be studied with profit, as one phase of the development of knowledge of plant nutrition. The interrelations of nitrogen and potassium have been given special attention. The quantitative importance of these two elements provides one reason for this.

The form of nitrogen supplied to the plant, whether as nitrate or ammonia, seems to influence the time at which potassium deficiency injury appears, when the availability of this element is in a range of deficient supplying power on the part of the medium. This has been explained by the theory that potassium is in some way essential to nitrogen transformations in plant metabolism. When potassium is deficient in the root medium and if ammonia enters the plant rapidly, the synthesis of some forms of organic nitrogen may fail and ammonia accumulate, with the production of severe and rapid injury to the tissues, as illustrated by experiments on tomato plants (WALL, 1940). High nitrogen in nitrate form may accelerate the injury produced when the potassium supply is deficient, within certain zones of deficiency, according to some observers. The whole question of nitrate and

potassium is further complicated by the fact that nitrate must be reduced and the role of potassium, if any, in this reduction is not understood.

The potassium supply has indirectly a relation to respiration of plant tissues. In the experiments of GREGORY's laboratory on barley plants low potassium was found to be correlated with increase of respiration, when sugar concentration in the plant was not too low. More specifically the respiration was correlated with protein content and also amino-acid content. High content of amino acid is present under some potassium deficiency conditions. GREGORY (1937), however, does not conclude that potassium is primarily associated with protein synthesis, but rather with the maintenance of the protoplasmic content. In the absence of sufficient potassium rapid proteolysis is thought to occur. STEWARD and PRESTON (1941), on the other hand, find in their experiments with potato discs that potassium absorption enhances respiration and protein synthesis. They worked with aerated potato tuber cells which under the experimental conditions were capable of growth and in which a progressive gain in protein occurred with the utilization of a substrate of amino acids. A contrast was found in this respect between these experiments and some of those conducted with leaves of grass plants. Other difficulties arise in comparing results of different investigators on leaf metabolism because the effects of age of the leaves are not always considered.

One of the outstanding difficulties of understanding what roles potassium plays, aside from a function in buffer systems, is that no indispensable organic combinations of potassium have been discovered and that nearly all potassium ordinarily exists in inorganic form. At one period many articles appeared on the possible function of the radio-activity of one of the naturally occurring isotopes of potassium. Little has been said of this hypothesis recently. The radio-activity is exceedingly feeble and no confirmed evi-

dence has been submitted that it has any part in physiological processes.

I have not been able to leave a clear impression of the functions of potassium in plant metabolism, and aside from the inadequacy of the discussion, this could not be accomplished. At one time or another a role in almost every important physiological process in the plant has been ascribed to potassium. The crucial experiments have not yet been performed that would enable us to say just how potassium enters into varied steps of organic transformations or how it influences the status of protoplasmic colloids. It is at present impossible to state definitely what peculiar chemical properties this element has that makes it indispensable to the physiology of plants and perhaps of all living organisms.

I have offered this outline primarily for the objective of illustrating the ramifications of research in an attack on any general problem of plant nutrition and the need of many approaches to proceed toward a goal of increased understanding of soil-plant interrelations.

REFERENCES:-

BRAY, R. H. and DE TURK, E. E. The release of potassium from non-replaceable forms in Illinois soils. Proc. Soil Science Society of America 3: 101-106, 1938.

GREGORY, F. G. Mineral nutrition of plants. Annual Review Biochemistry VI: 557-578, 1937.

HARTT, CONSTANCE ENDICOTT. Potassium deficiency in sugar cane. Botanical Gazette 88: 229-261, 1929.

HOAGLAND, D. R. and MARTIN, J. C. Absorption of potassium by plants and fixation by the soil in relation to certain methods for estimating available nutrients. Trans. of Third International Congress of Soil Science 1: 99-103, 1935.

—— —— —— ——. Absorption of potassium by plants in relation to replaceable, non-replaceable, and soil solution potassium. Soil Science 36: 1-33, 1933.

JENNY, H. and AYERS, A. D. The influence of the degree of saturation of soil colloids on the nutrient intake by roots. Soil Science 48: 443-459, 1939.

JOFFE, J. S. and KOLODNY, L. The effect of alternate drying and wetting on the base-exchange complex with special reference to behavior of the K-ion. Proc. Soil Science Society 3: 107-111, 1938.

JOHNSTON, EARL S. and HOAGLAND, D. R. Minimum potassium

level required by tomato plants grown in water cultures. Soil Science 27: 86-109, 1929.

LILLELAND, O. Experiments in K and P deficiencies with fruit trees in the field. Proc. Amer. Soc. Hort. Sci. 29: 272-276, 1932.

—— —— and BROWN, J. G. The potassium nutrition of fruit trees, I. Soil Analyses. Proc. Amer. Soc. Hort. Sci. 35: 327-334, 1937.

—— —— —— —— . The potassium nutrition of fruit trees, II. Leaf Analyses. Proc. Amer. Soc. Hort. Sci. 36: 91-98, 1938.

NIGHTINGALE, G. T., SCHERMERHORN, L. G. and ROBBINS, W. R. Some effects of potassium deficiency on histological struc- ture and nitrogenous and carbohydrate constituents of plants. N. J. Agr. Exp. Sta. Bull. 499: 1-34, 1930.

PAGE, J. B. and BAVER, L. D. Ionic size in relation to fixation of cations by colloidal clay. Proc. Soil Science Soc. 4: 150- 155, 1939.

PIRSON, ANDRE. Über die Wirkung von Alkali-ionen auf Wachs- tum und Stoffwechsel von *Chlorella*. Planta, Archiv für wissenschaftliche Botanik 29: 231-261, 1939.

RICHARDS, F. J. Physiological studies in plant nutrition, III. Further studies of the effect of potash deficiency on the rate of respiration in leaves of barley. Annals of Botany 46: 367-388, 1932.

SCHACHTSSCHNABEL, PAUL. Aufnahme von nicht-austauschba- rem Kali durch die Pflanzen. Zeitschrift f. Bodenkunde und Pflanzenernährung 48: 107-133, 1937.

STEWARD, F. C. and PRESTON, G. The effect of salt concentra- tion upon the metabolism of potato discs and the contrasted effect of potassium and calcium salts which have a common ion. Plant Physiology 16: 85-116, 1941.

WALL, MONROE E. The role of potassium in plants III. Nitro- gen and carbohydrate metabolism in potassium-deficient plants supplied with either nitrate or ammonium nitrogen. Soil Science 49: 393-409, 1940.

PLATE 1. — Illustration of the effects of continuous fallowing or cropping on the ability of representative Californian soils to support a crop of barley. Also illustrates general nature of installations used in investigations of soil solution phenomena.

In foreground 13th successive annual crop of barley cropped soils ("A" soils).

In background 2nd successive annual crop of barley following a 10-year fallow period ("B" soils). Only part of tanks shown. (From BURD and MARTIN, unpublished).
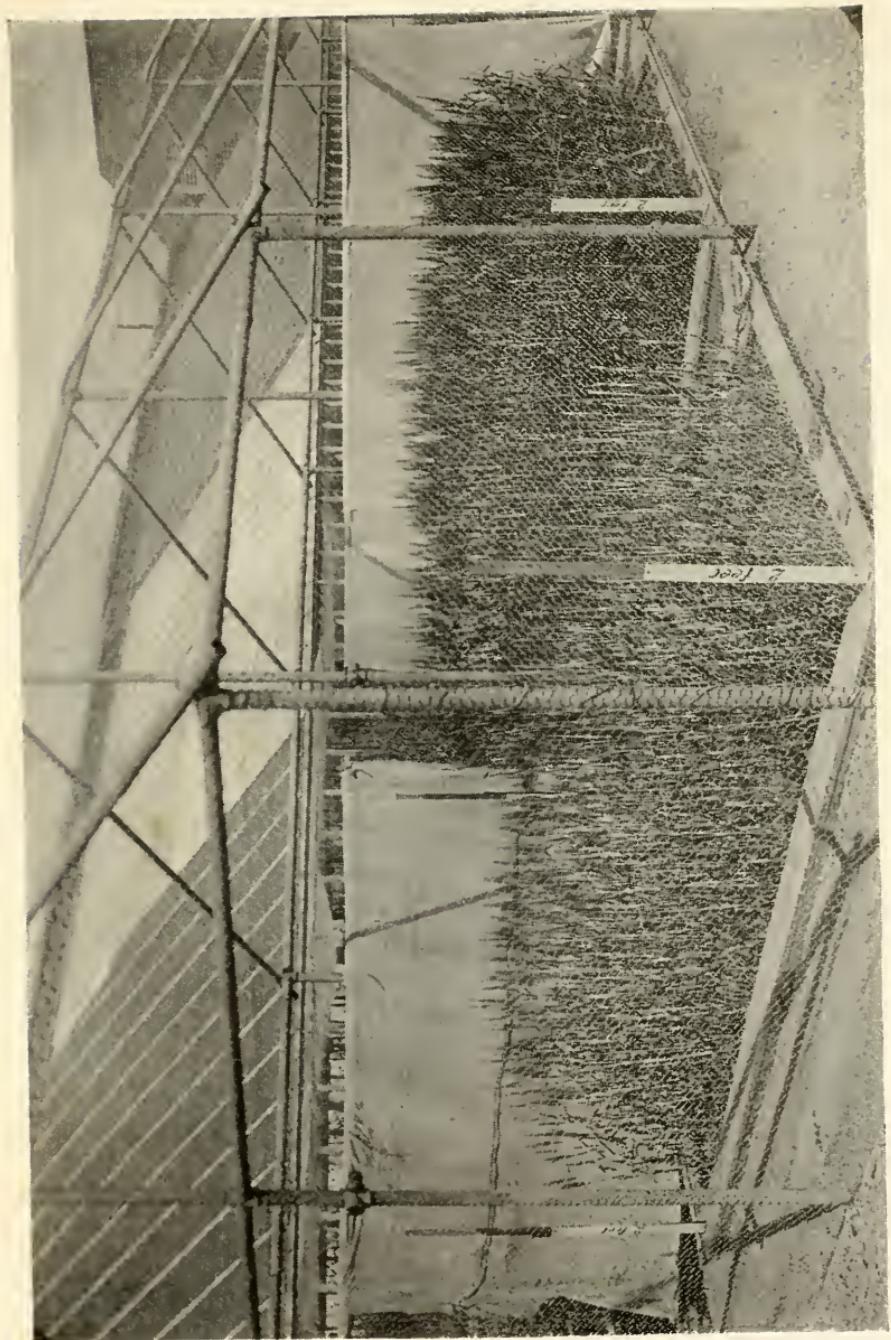
PLATE 2. — Soil displacement apparatus, consisting of a battery of brass tubes, 17 inches length and 3 inches diameter. A brass screen rested on the bottom of each tube. Soil is packed very carefully in the tubes and water placed on top of the column of soil. Air pressure is applied as necessary to effect dis- placement of soil solution. (From BURD and MARTIN, unpublished).
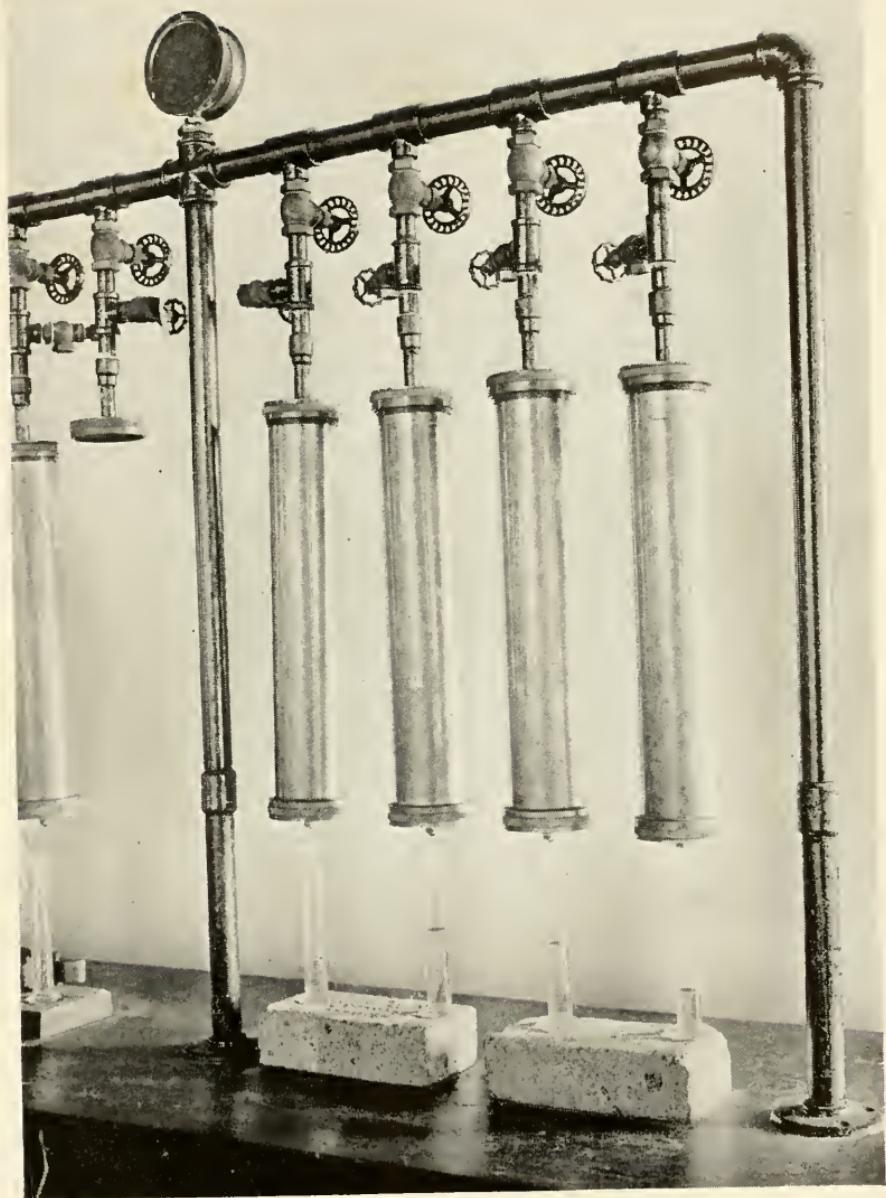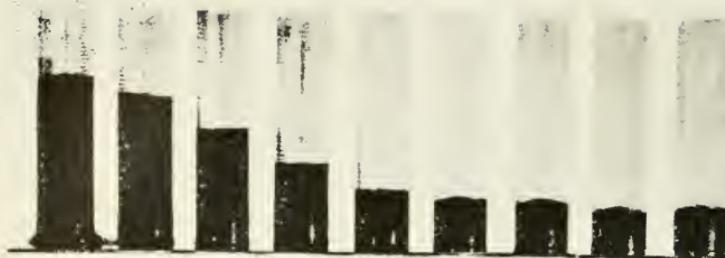
Na.	100	75	50	40	30	20	10	5	0
Ca.	0	25	50	60	70	80	90	95	100
cc.	7.20	6.50	5.08	3.20	2.30	2.10	2.00	1.95	1.86

PLATE 3. — Settling volumes of soil containing in its colloidal fraction varying proportions of replaceable sodium and calcium. Observe large effects of sodium versus calcium saturation (left and right cylinders). (From undetermined source).

PLATES 4 and 5 (next two pages). — Plot 23. Alfalfa, no treatment.— Plot 24. Alfalfa, sulphur plot. — An experiment in the field demonstrating reclamation of a black alkali soil. In this case sulphur was used to provide acid by oxidation to liberate Ca ions from carbonates and displace sodium ions held by soil colloids. Other methods have also been employed successfully. (See discussion by KELLEY in Calif. Agr. Exp. Sta. Bulletin 617). (From W. P. KELLEY, 1937).
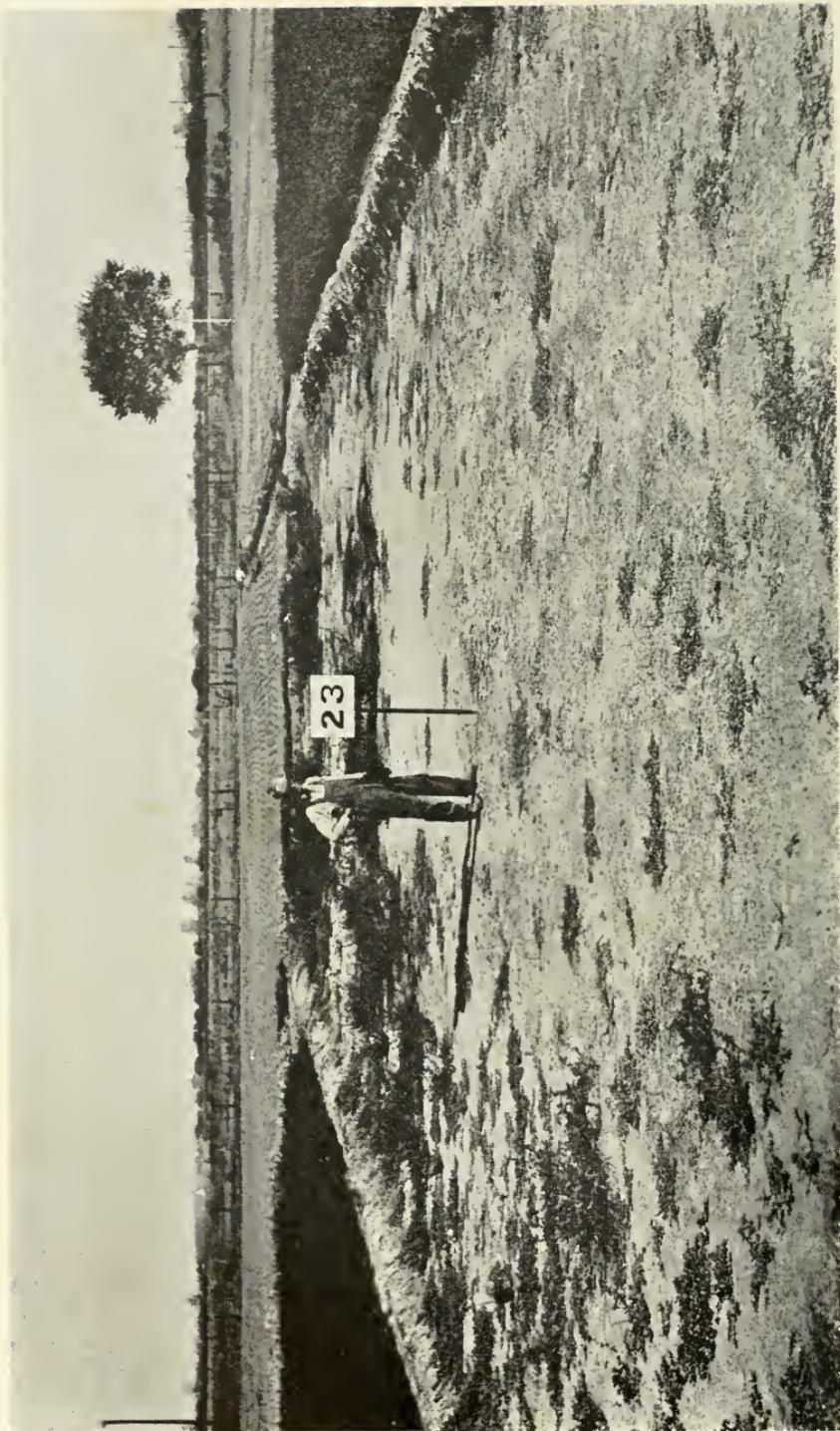
— PLATE 4 (for legend to plates 4 and 5, see previous page) —
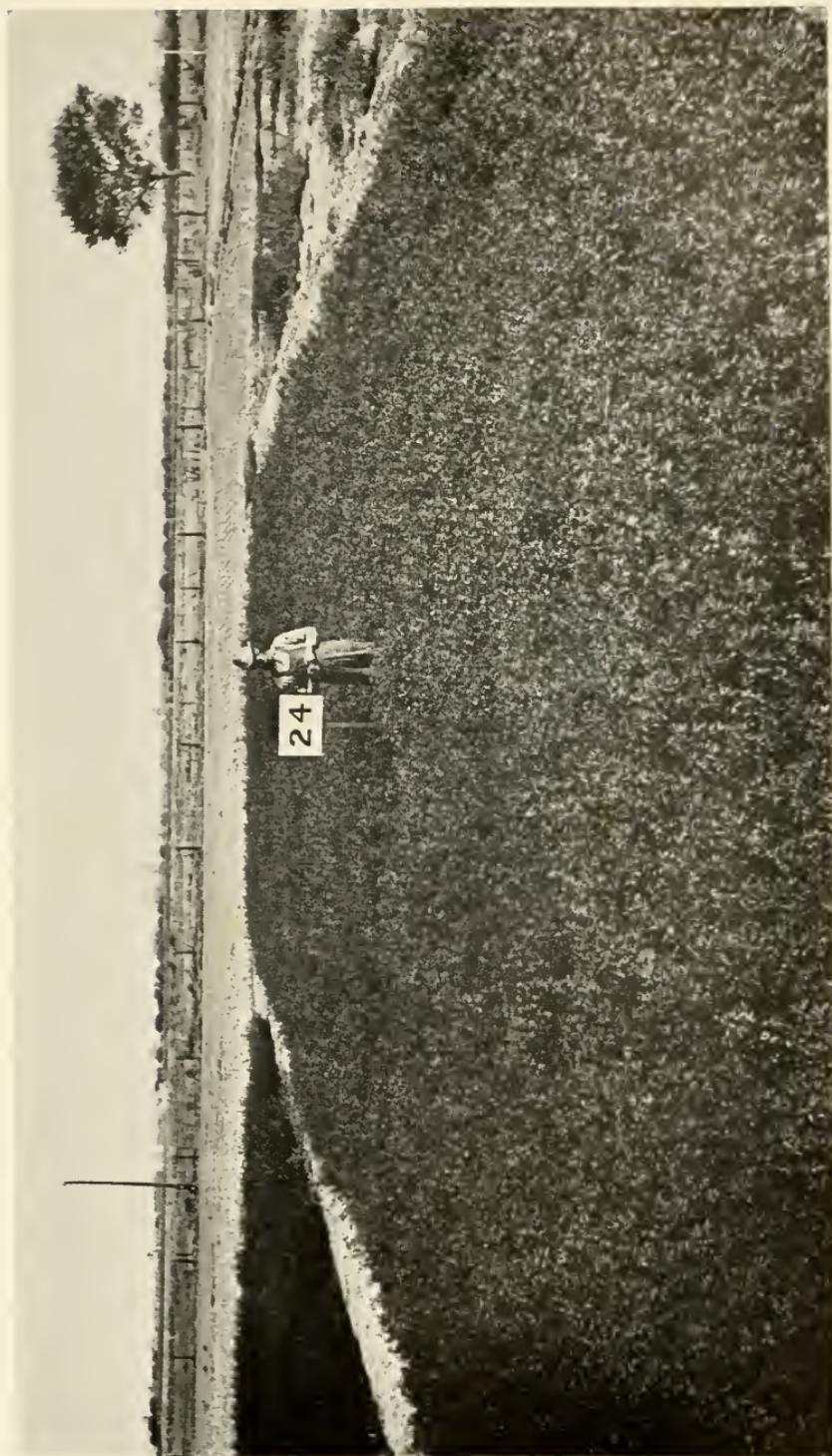
— Plate 5 —

PLATE 6. — Control chamber 5 feet by 2½ feet, employed for growing plants under specified conditions of light (artificial), temperature and humidity. The apparatus is air conditioned with the aid of a refrigerator unit of large capacity. In the experiment illustrated above fluorescent lights form the source of illumination. In earlier investigations with young wheat plants Mazda lamps were employed. Control of the root environment is gained by furnishing to the roots the desired nutrient solution. Air movement is governed but measures for working with different concentrations of CO_2 in the atmosphere have not yet been developed. Two chambers are at present available. (This illustration provided by K. A. GROSSENBACHER).
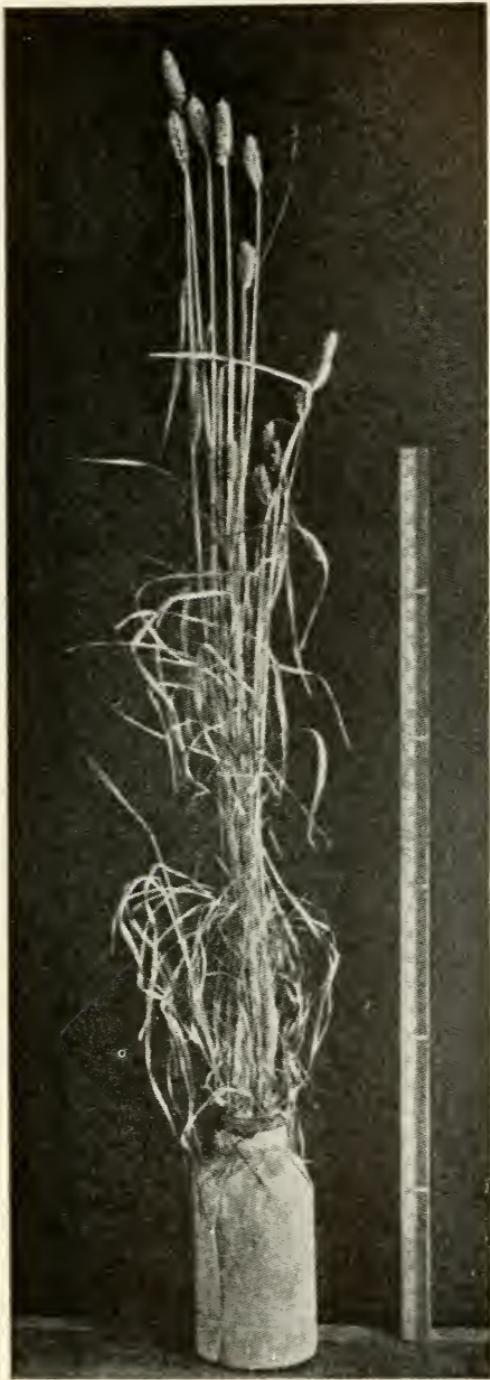
PLATE 7. — Wheat plants (13 weeks from time of planting) grown throughout their cycle in chambers under artificial light, with temperature control. Mazda lights were used. (From DAVIS and HOAGLAND, 1928).

PLATE 8. — Leaves from tomato plants grown in nutrient solutions. Left, copper deficient. Middle, complete solution. Right, zinc deficiency. (Courtesy of D. I. ARNON).

PLATE 9. — Plum seedlings grown in nutrient solutions, showing appreciable requirement for copper. .01 part per million of copper in the solution was entirely inadequate under these culture conditions. (From HOAGLAND, 1940).
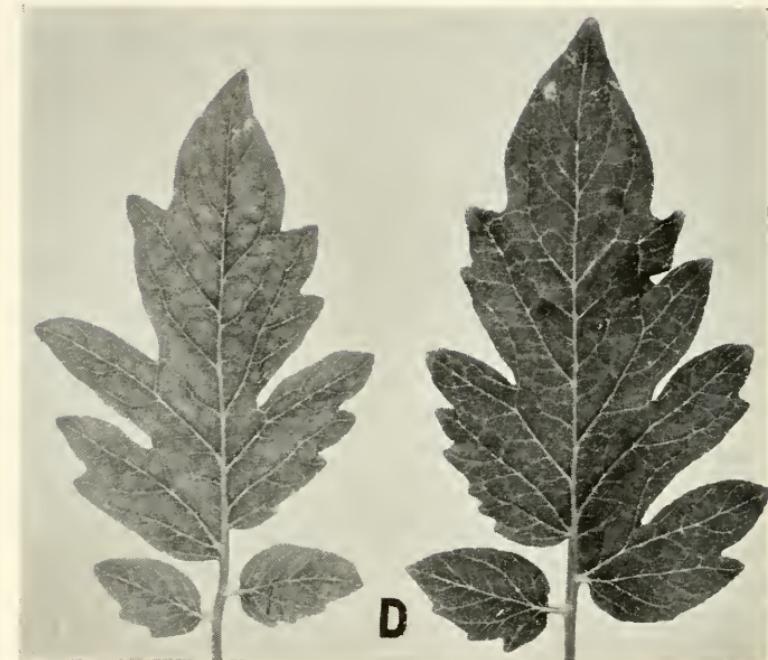
PLATE 10. — Leaves from tomato plants grown in nutrient solutions. Left, no molybdenum added. Right, complete solution, including molybdenum. (From ARNON and STOUT, 1939).

PLATE 11. — Plum seedlings grown in culture solution. Effect of omitting molybdenum from the solution. (From HOAGLAND, 1940).

PLATE 12. — Apricot seedling grown in nutrient solution in pyrex vessel, under zinc deficiency condition. Zinc deficiency symptoms were similar in several respects to those shown by seedlings growing in soil producing the little-leaf disease. (From HOAGLAND, CHANDLER and HIBBARD, 1936).

PLATE 13. — Corn plants growing in a soil (subsoil) naturally deficient in zinc supplying power for various tree species.

In the greenhouse corn plants develop "white-bud" symptoms. Soil on left—no treatment; next, soil previously autoclaved; soil autoclaved and reinoculated with unsterilized soil; soil similarly treated but to which zinc was applied. This experiment was frequently repeatable but did not always succeed. Uncontrolled inoculations could not be avoided. The hypothesis is that the growth of certain kinds of microorganisms may have interfered with the absorption of adequate amounts of zinc by the corn plants, possibly through competition for zinc.

Minus zinc | 005 PPM zinc | 01 PPM zinc | 02 PPM zinc | 05 PPM zinc | 1 PPM zinc

119-G
3/2/38
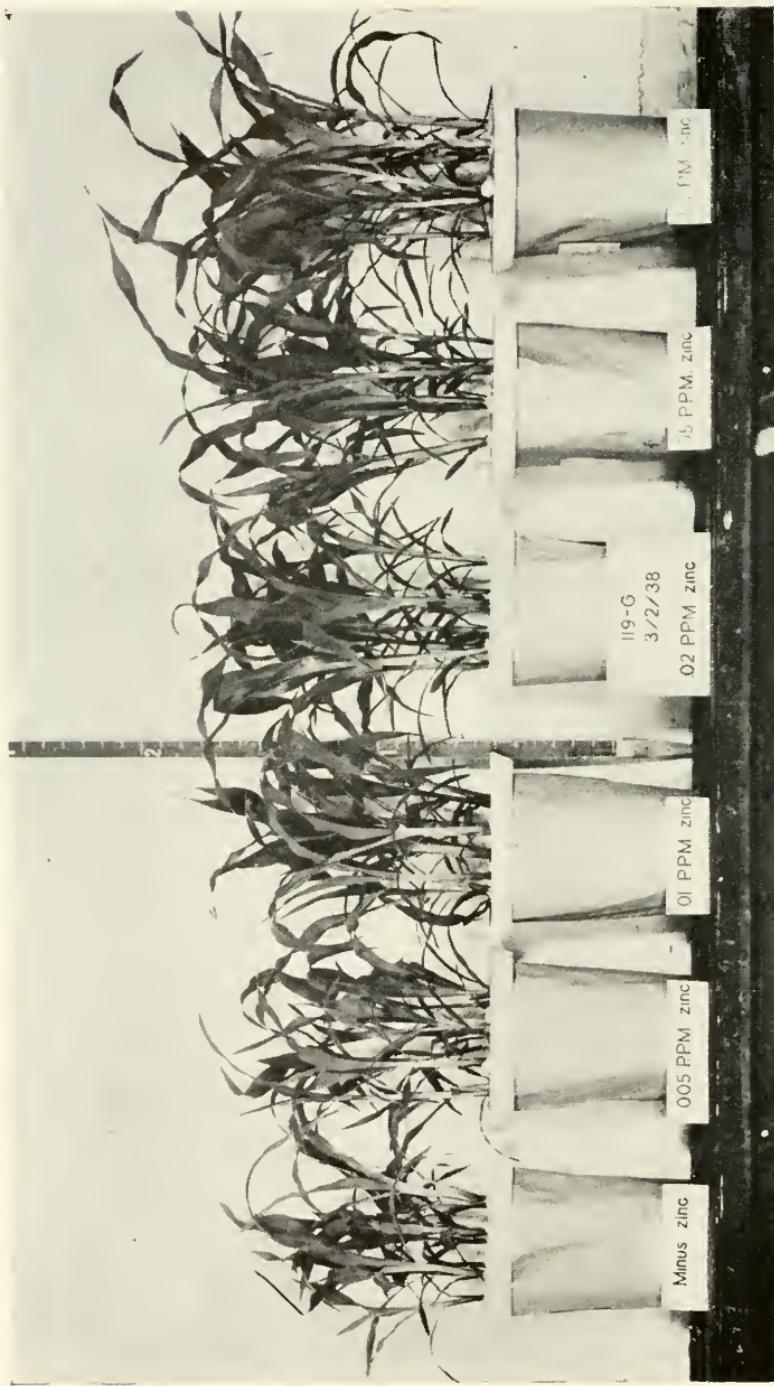
— PLATE 14a —

PLATES 14a AND b. — Corn plants grown in greenhouse in the same nutrient solutions, containing graduated amounts of zinc, at two seasons of the year. Plate 14a: set harvested March 2, and plate 14b: June 6. Illustrates effects of climatic condition, particularly light, on responses to zinc deficiency. (From STOUT and HOAGLAND, unpublished).
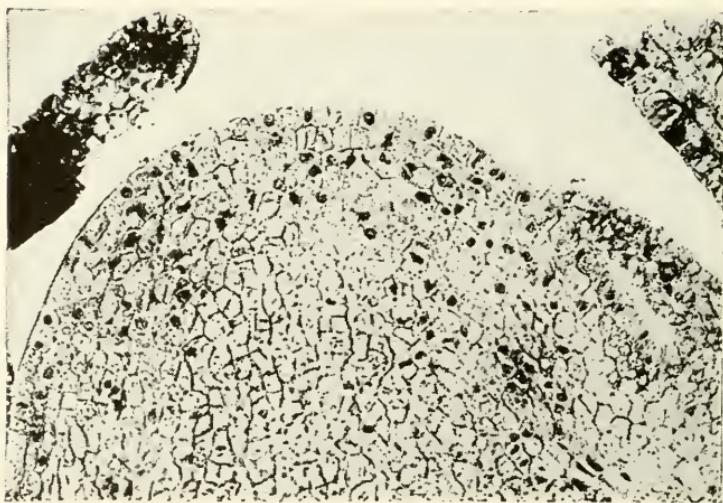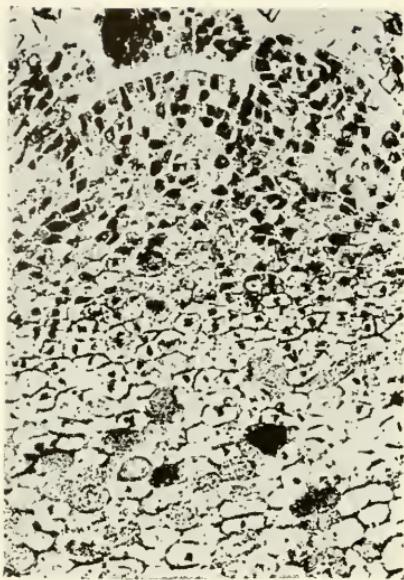
PLATE 15. — Growing points of apricot buds. *Top,*
from zinc deficient tree. *Bottom,* from normal tree.
(Courtesy of Dr. H. S. REED).

PLATE 16. — Photograph of cells of *Nitella clavata*. Cell sap but slightly contaminated can be obtained from long internodal cells, which reach a length of several inches.
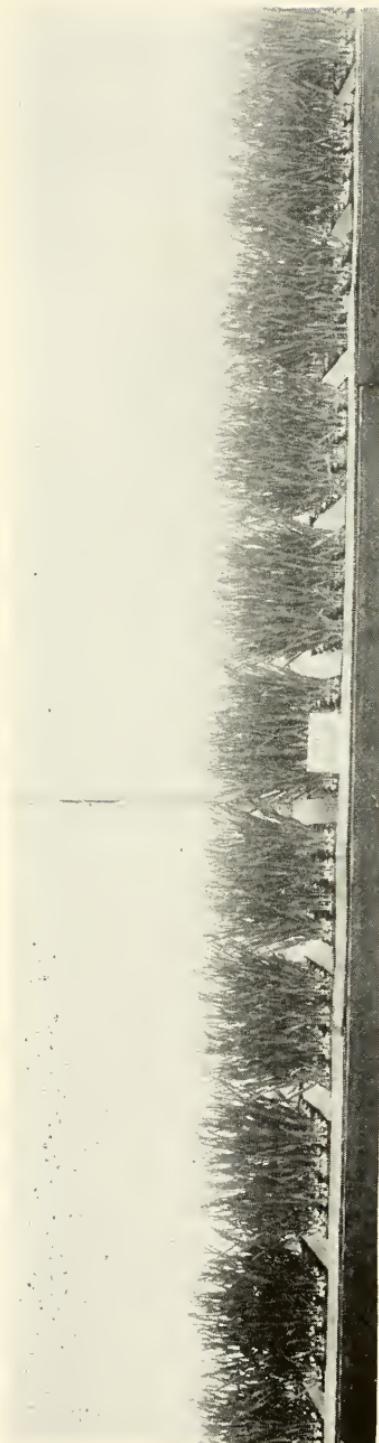
PLATES 17 AND 18. — Illustrations of a method of growing uniform sets of barley plants to obtain ex-cised roots for absorption studies; 168 three-week old plants in each set. (From HOAGLAND and BROYER, 1936).
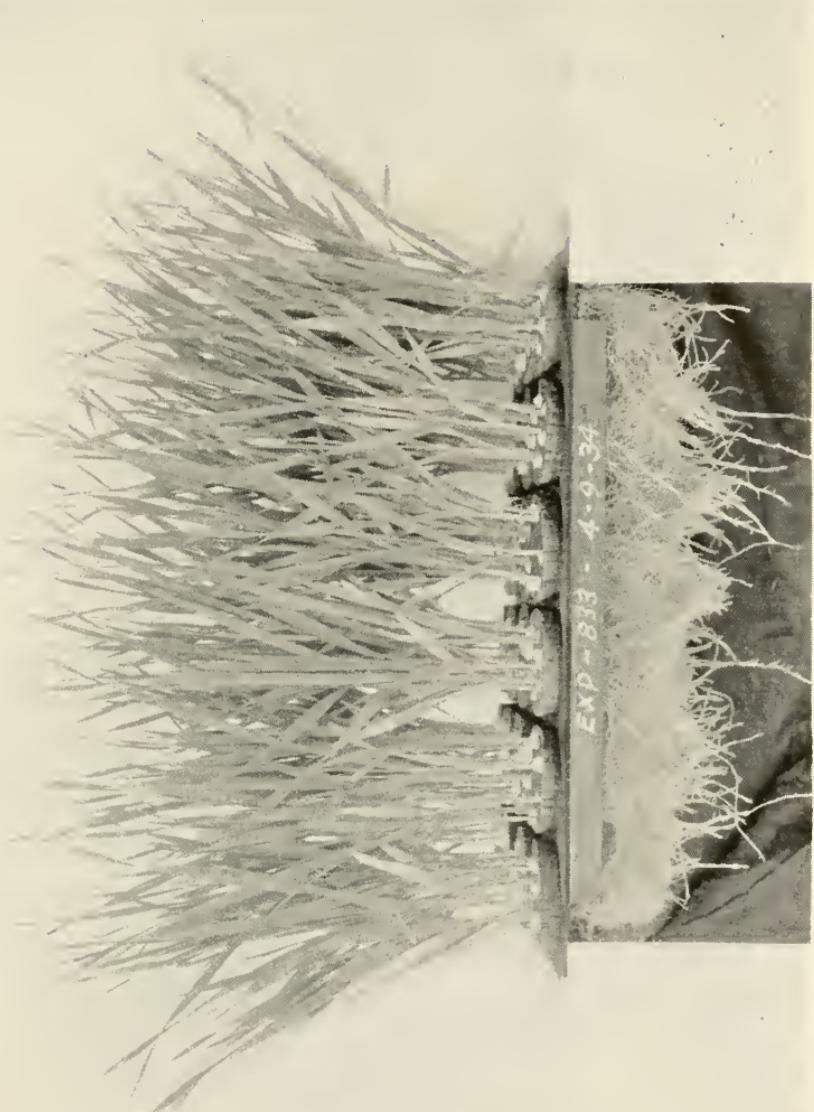
— PLATE 18 —

PLATE 19. — Illustrating a radiograph of plant tissue. — A contact radiograph of a young leaf removed from the plant 36 hours after introducing the P*O₄ into the nutrient solution. The time of exposure was one hour. The light areas, *a*, *b*, *c*, *d*, were caused by folds in the leaves and *e* by the bunching of several small leaflets. (From ARNON, STOUT, and SIPOS, 1940).

PLATE 20. — Growth of tomato plants in three types of culture; soil, sand, and solution. All cultures yielded at the same order of magnitude, when grown side by side in the greenhouse. All yields of fruit were very large. (From ARNON and HOAGLAND, 1940). The solution culture technique was that devised by W. F. GERICKE.

PLATE 21. — Various types of vessels used for nutrient solution experiments, ranging from large tanks, 10 feet long, to small pyrex baking dishes. The tanks are made of welded black sheet iron— coated on the inside with non-toxic asphalt paint and on the outside with asphalt paint and a coat of aluminum paint. The choice of container depends on the objective of the experiments; size of plants to be grown, necessity for maintaining purity of culture, density of spacing of plants, kind of aeration, etc.
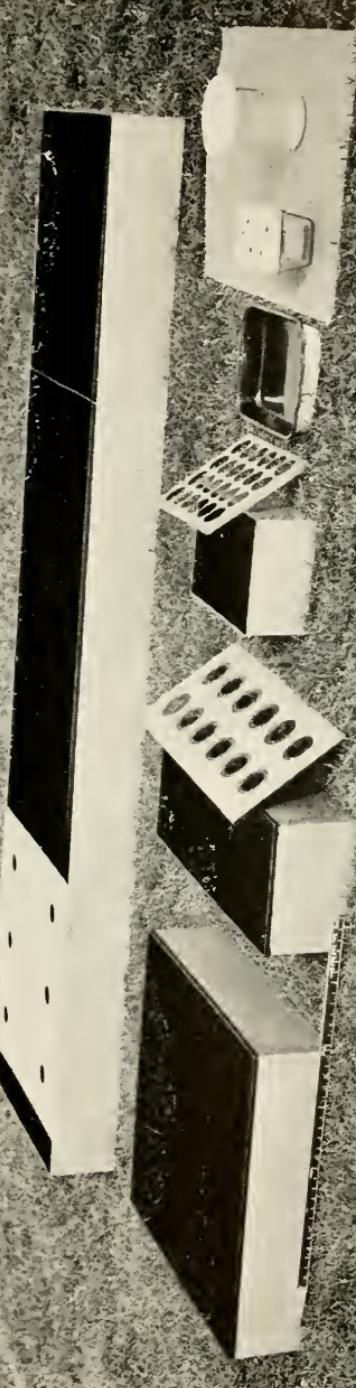
PLATE 22. — Culture installation for growing lettuce plants in aerated nutrient solutions. (From experiments of D. I. ARNON).

PLATE 23. — Lettuce plants grown at va-
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lished).
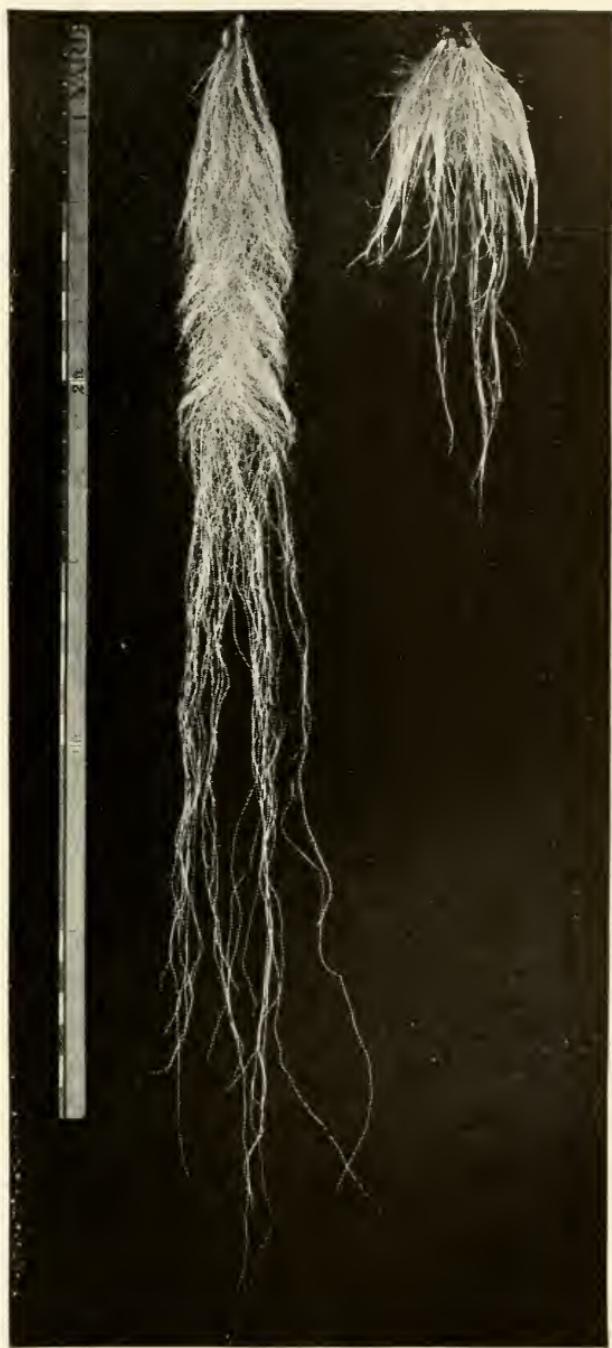
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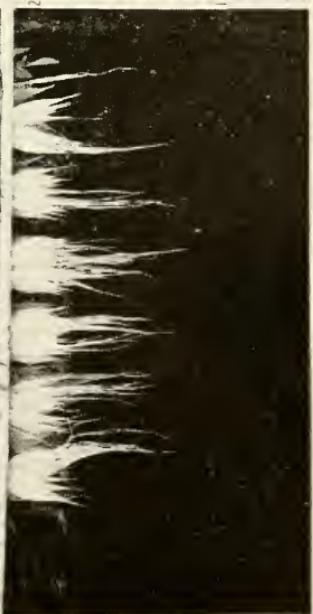
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