MINERAL NUTRITION OF PLANTS

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For bibliographical reasons, Professor Emil Truog, chairman of the committee responsible for this book, has been designated editor. Listing volumes of essays written under separate authorship presents problems to bibliographers which do not easily lend themselves to practical solution. The Press, therefore, feels that scholars will be grateful for a simple entry under which this book may appear in files, catalogues, and bibliographies.

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Preface

Mineral nutrition of plants is a subject of tremendous interest and importance to many people. The plant physiologist must, of course, devote much of his attention to this field; the agronomist, horticulturist, and forester meet problems almost daily which require for their solution specific knowledge dealing with the mineral nutrition of many kinds of plants; and lastly, the fertilizer manufacturer, who is called upon to supply the needed mineral nutrients when they are lacking in soils, must, through his technical expert, keep informed of the latest findings in this field if his enterprise is to attain and maintain a forefront position.

Because these people representing several different but related lines of activity seldom have a suitable opportunity as a group to discuss those phases of the mineral nutrition of plants which are of common interest and concern, Mr. D. D. Long, technical adviser in the fertilizer industry, suggested that a symposium be held so that the latest information, both theoretical and practical, associated with the mineral nutrition of plants might be presented by leaders in the different but allied fields concerned. Accordingly, a committee of eleven people representing six national scientific societies and three other agencies dealing with technical matters in this field was organized to plan and arrange such a symposium. Broad representation on the committee gave assurance that the symposium would be national in scope. Sixteen institutions and agencies located in areas from coast to coast, and one each from Hawaii and Sweden, were represented by speakers on the program. More than five hundred persons attended the meetings; ten foreign countries were represented.

The Committee expressed a desire to hold the symposium at the University of Wisconsin, because of its central location. This University was particularly happy to accept this proposal; the suggestion came at the time the University was making plans for celebrating its centennial during the year 1948-1949, and in this connection it was serving as host to a number of symposiums. Fortuitously, this one in Madison was a "natural."

In arranging the program, the Committee made an attempt to provide a logical sequence of papers, starting with the soil—the natural source of mineral nutrients—and then treating successively the subjects of entry and translocation of mineral nutrients in plants, the role of minerals in plant nutrition, and finally such modifying influences as light and soil moisture. Two special papers on more practical aspects of the subject were included. Although it was, of course, not possible to treat all phases of this rather broad subject, and many gaps were necessarily left, yet the up-to-date information provided by the contributors, all of whom are actively engaged in research, will be of special interest and value to many teachers and investigators, as well as to others concerned with the practical applications. One of the primary objectives of the symposium was to provide an opportunity for presentation of latest views regarding the availability of mineral nutrients in soils and mechanisms of absorption and translocation of these mineral nutrients by plants. The papers on these topics should be of special interest.

The necessary financial support was provided through funds of the Wisconsin Alumni Research Foundation administered by the Graduate School of the University of Wisconsin and by a special grant from the National Fertilizer Association. Grateful acknowledgement is made to these sponsors and to all who took part in the program and served on committees. Especial thanks are due to Drs. L. E. Englebert, Gerald C. Gerloff, and Folke Skoog for invaluable help in reading proof.

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EMIL TRUOG

Table of Contents

MINERAL NUTRITION OF PLANTS

PHYSICO-CHEMICAL AND BIOLOGICAL FACTORS AFFECTING NUTRIENT AVAILABILITY IN SOILS

MECHANISM OF ENTRY AND TRANSLOCATION OF MINERAL NUTRIENTS IN PLANTS

SOME FIELD PROBLEMS IN PLANT NUTRITION

ROLE OF MINERALS IN PLANT NUTRITION

MODIFYING INFLUENCES OF VARIOUS ENVIRONMENTAL FACTORS UPON MINERAL NUTRITION

MINERAL NUTRITION OF PLANTS

CHAPTER

1

World Food Possibilities and Fertility Status of Our Soils

CHARLES E. KELLOGG and A. C. ORVEDAL

MANKIND has lived for a long time on the soils of the world. Despite the examples of spectacular soil depletion that each of us has seen or read about, we still must marvel at the stability of our soils. For many centuries, long before the rise of modern science, eastern and southern Asia had enormous populations. Famines have occurred, to be sure; in fact, this population has rarely been well fed by modern standards, yet it has persisted through the ages. Both western Europe and Japan have maintained increasing populations for three centuries on soils originally low in fertility—soils that have been greatly increased in productivity during the last century and a half.

What hope, in the light of modern science, can we now give to man that hunger and starvation can be kept from his door? Realizing fully the many critical social, economic, and political obstacles to be overcome (3), what about soils and crop production? Let us look broadly at a few of the physical and biological aspects.

ARABLE LAND—AVAILABLE AND POTENTIAL

First, do we have enough arable land in the world? If we take a general view of the world's land, we see that about one-half of it is not suitable for cultivation (5). This includes areas covered with everlasting ice and snow, the Tundra, the high mountains, the deserts, and semideserts. There is some significant grazing in the high mountains, and water collected in the high mountains is used for irrigating desert lands. In fact, substantial increases in irrigation are possible with modern methods of soil classification, irrigation, drainage, and fertilization.

The other one-half of the world's land is only partly arable. Some soils are too stony, too sandy, too hilly, too salty, or too wet for cultivation. A sharp line cannot be drawn between those which are arable and those which are not. First, any estimate must be based on economic conditions, either consciously or unconsciously assumed. It is physically possible to grow crops almost anywhere: mountainsides can be terraced; stones can be removed; dikes can be built; and water can be carried long distances. Secondly, we do not have a good soil map of the world. Soil maps for several large areas do not exist. In these we can only fall back on informed opinion. Thirdly, the potential use of soil depends upon the associated industrial facilities and transportation.

Even estimates of the land now cultivated in the world vary widely around ten per cent. This is because we may start with intensive cultivation and pass gradually through general farming and extensive farming to nearly wild land with no sharp breaks.

Western civilization—our civilization—has grown up mainly in the temperate regions, first in Europe, then in America, and later in Australia, New Zealand, South Africa, and similar places. It began on the well-watered forested soils near the oceans and great rivers. With the development of technology, it has spread to the interior of continents along the railroads. Now most, although by no means all, of the good land in temperate regions is occupied. We could expand considerably in the United States, probably to a total of around 450 million acres, or possibly even 500 million under conditions of reasonably full employment. This does not count some of the poor soil now in farms that should be used for ranching and forestry.

North of the temperate region in the region of Podzol soils, only about one per cent of the land is cultivated. If we increased the percentage to ten, about 300 million acres of new soil would be available. On the basis of experience in Finland and Scandinavia this seems reasonable, provided transportation and industry are developed along with the agriculture. At least when first cultivated, these soils would not be so fertile as most of those in the temperate region, but experience has demonstrated that they are responsive to management and can be well developed for dairying and for vegetables, including potatoes.

The great areas of undeveloped soil in the world are in the tropical regions in Africa, South America, Central America, and several of the great tropical islands. In southeastern Asia and India, on some of the Pacific Islands, and in a few parts of other tropical regions, these soils are now intensively used for crops. Yet there are great areas that are hardly touched in relation to their potentialities. It seems reasonable to suggest that at least twenty per cent of the unused tropical soils in the Americas, Africa, and the great islands like New Guinea, Madagascar, and Borneo could be cultivated. This would give us approximately one billion additional potential acres. If we estimated the potential productivity of these tropical soils on the basis of the best results, say in Hawaii and Java, the figure would be almost astronomical. It would be conservative to use experience in the Philippines as a guide, realizing when we do so that this omits consideration of the great potential increases in efficiency that could come with the application of modern science in the tropics to the same extent we have applied it in the temperate regions. For the northern soils, the 300 million acres north of the temperate region, we may use Finland as a guide.

Calculations indicate that with this new land we could more than meet most items listed in the world food needs for 1960 by the Food and Agriculture Organization of the United Nations. A few in short supply could easily be increased through some shifts of the agricultural pattern (*6*).

This estimate of 1,300,000,000 acres is probably either too low or too high. A satisfactory estimate cannot be had now. When checked with a reliable soil map, which we may have in a few years, the results will depend a great deal upon the economic assumptions used. Of course, we shall never have an exact figure. At any rate, a large potential acreage exists.

It must be emphasized that these new acres will be "difficult" acres in contrast to some of the land settled by Americans in the past 100 years. There is very little new soil in the world simply waiting for the plow. Most of these new acres will require clearing. They will need careful management from the start. Some of the soils require terraces, some levees, some partial drainage, and some need supplemental irrigation during dry seasons. A large part will need lime, fertilizer, or both from the start.

Most of these acres are in the interior of continents, away from regular trade routes or far from good harbors. In order to use them, medical facilities, local industry, and electric power must go along with agricultural development. Families settled by themselves or in small groups in the Middle West and in the Great Plains during the nineteenth century. Transportation, industry, and the other services followed them. Very few of these prospective new acres can be settled in this way. On them settlement shall need to be planned with the idea of combined resource development: water, soil, forest, minerals, and power must be considered together.

We do not really need all this new land—not yet. During the war careful estimates made by the Department of Agriculture and the land grant colleges showed that it would be entirely practicable to increase agricultural production in this country by about twenty per cent on most items and higher than that on several (*8*). Somewhat lower increases might be expected in some other countries, but in most of them considerably higher ones could be had through the application of what is now known. Thus, even without any new soil the food needs of the world could be met for cereals, roots and tubers, and sugar. But some new areas or further increases in yields beyond those assumed in this earlier study would be needed to supply a bit more fats and oils and considerably more pulses and nuts, fruits and vegetables, meat, and milk.

Thus, taking the two together—the potential new soil and the demonstrated potential production under good management on land already being farmed—the world could have food far beyond the amounts estimated as required for the world population in 1960.

Now, such estimates may appear to be very optimistic. They probably are in terms of the real political and economic situation that we see about us. They indicate what *could* be done with our present knowledge *if* adequate institutional arrangements were made for effective soil use on a sustained production basis. In another sense they are low. They are low because these estimates take no account of entirely new technology. They assume merely a general acceptance of existing technology as now used by millions of farmers. Yet we know that the efficiency of farm production is increasing at an accelerated rate. It has increased considerably since the estimates were made by the Department of Agriculture and the land grant colleges.

And we must recall again that the level of production we are seeking will not be based upon history, nor based upon any mythical "natural balance." *It is that level of production on a sustained basis made possible by modern science and technology in a peaceful world with reasonably full employment.* We seek an effective cultural balance between people and resources.

We in the United States and in western Europe have been inclined to take for granted the enormous increases in efficiency of production during the past 150 years. These have been reflected in yields for a long time in Europe although not in the United States until recently. There are several reasons for this difference. In the United States land has been relatively plentiful and labor relatively expensive, except during periods of depression. Alert farm managers are concerned as much with reducing inputs as with increasing outputs. Many of our most important improvements are designed to reduce labor even at some sacrifice of total harvest. During the latter part of the nineteenth century and the early part of the twentieth century, many millions of acres of new soil were brought into use in the subhumid and semiarid regions where normal yields are relatively low. Then, too, many of our plant breeding programs have been concerned with widening the range of soil types on which important crops may be grown rather than simply increasing the yields on a few soil types. As a result, our farmers have many more choices of crops than they had formerly. Some farmers have allowed their soils to deteriorate seriously, even while successful farmers were improving theirs.* Yet even so, there have been

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*Erosion, decline of organic matter, increasing acidity, loss of soil structure, increasing salinity, soil blowing, and especially declining plant nutrients, are all in the picture. However, there has also been improvement on many farms through the use of lime on soils naturally acid, the use of fertilizers on soils naturally low in plant nutrients, drainage, irrigation, the introduction of legume meadows, and livestock farming. No one knows what the net result has been. Nor would it make much difference to these estimates if we did know, since our efficiency is so much higher than it was 200 years ago and so much lower than it could be significant increases in the average yields of several of our major crops in the last few years, aside from climatic effects.

As applied to the United States, these effects of technology are much more clear when considered in terms of efficiency (2). For example, between 1800 and 1940 the number of man hours needed to produce 100 bushels of wheat dropped from 373 to 47. Similar improvements were made with the other major crops. In 1820, after science had already had some effect, one farm worker supported about four and one-half other people. In 1946 the figure was fourteen and one-half.

Modern science has already increased our efficiency and continues to do so at an accelerated rate. This has happened in the temperate regions. There is every reason to believe that similar potentialities for the application of science to agriculture exist in the far north and especially in the tropics. The tropics have some handicaps but they also have many advantages. Our guess would be that science will be even more influential in the tropics than it has been in the temperate regions. The great need there is for research institutes devoted to fundamental investigations. The soils are so different from those in the temperate regions that technology can be transferred only to a very limited extent.

SOIL FERTILITY OF THE GREAT SOIL GROUPS

Following this general view of the potentialities for food production, let us look more specifically at soil fertility. Plant nutrients tend to become limiting wherever man uses soils for crops, but the fertility problems vary in both kind and intensity from place to place. Different crops have unlike nutrient requirements, and different soil types vary in their capacity to provide nutrients. Since many thousands of unique soil types exist in the world, exceptions must be permitted in any generalization. Yet, a broad view of the fertility status of the principal great groups of soils may help us measure the fertility problems in world food production.

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with what we know now. Of course, we do need to learn these relationships specifically, farm by farm, to get on with the job of preparing recommendations for sustained production.

Gray-Brown Podzolic soils

The Gray-Brown Podzolic soils are dominant in the agriculture of the United States from Minnesota east and Tennessee north, and in the northwestern part of Europe. Like other podzolic soils, these are leached, acid, and relatively low in most plant nutrients in available form. They are also rather low in organic matter. Despite these fertility deficiencies, they are highly responsive to management. With lime and fertilizers, they support a very wide range of crops. We have learned to make from them fertile, arable soils that give high yields.

Phosphatic fertilizers and lime are needed on nearly all of these soils. The amounts of potassium and nitrogen required depend upon the legumes grown and the animal manures available; but, usually, at least some chemical fertilizers containing these nutrients are required for optimum efficiency. In many places magnesium and boron are needed for some crops. Responses have been reported for most of the nutrients, but, generally speaking, deficiencies of the minor elements are less common than on the soils of warmer regions.

Western civilization grew up on the Gray-Brown Podzolic soils and expanded from them onto others. Most of the research on soils and soil-plant relationships, especially during the nineteenth century, was conducted on these soils and their close relatives. The results have been dramatic. From the fall of Rome nearly to the French Revolution, grain yields in Europe were around six to ten bushels to the acre. With the adoption of crop rotations, they nearly doubled. Not only that, the elimination of the fallow year gave more acres for harvest. The application of chalk and farm manures became general. In Germany, wheat yields went to about 16 bushels to the acre by 1850. At the same time, they were 14 bushels to the acre in France and somewhat over 20 bushels in Britain. By 1906, they had gone to 30 bushels in Germany and to over 30 in Britain, while they were about 20 in France. Now they stand at about 35 in Britain (*4*). Something more than one-half of the increases in Britain and Germany came before the common use of chemical fertilizers after 1850.

This remarkable increase in production has been attained through the use of improved techniques, most of which have grown out of scientific research over the last 150 years. On no other great soil group has a comparable amount of research been done and on no other can we find similar, widespread increases in production, although there are important advances in other areas that point to the potentialities.

Scientists and farmers alike, on the Gray-Brown Podzolic soils of Europe and the United States, know how to overcome the handicaps of relatively low fertility by liming, fertilization, growing legumes, and so on. The question of what yields to strive for is, within limits, an economic matter of input–output relationships of the various factors involved, including fertilizers. Yet, even now, only a part of the farmers on these soils are really efficient. If all of them followed the practices of the most efficient one-quarter or one-third, production could probably be increased another 25 to 35 per cent, to say nothing of possible contributions of entirely new technology.

Chernozems

The Chernozems are the great wheat-producing black soils of the United States, Canada, Argentina, and the Soviet Union. Unlike the Gray-Brown Podzolic soils, the Chernozems are highly fertile at the start. They have abundant organic matter and an excellent granular structure. They are rich in nitrogen, calcium, potassium, and most other plant nutrients.

In spite of this storehouse of nutrients, yields on Chernozems are not especially high, on the average. Rainfall on these soils is erratic and often low. So far, science has had conspicuously less success in overcoming the limitations set by drought than those set by low fertility. Still, a lot has been done recently to increase yields on Chernozems, especially through improvement in varieties of grains and through better and more timely tillage with powerful machines.

Yet, there are fertility problems with Chernozems. Under the prevailing practice of continuous grain production, the nitrogen supply declines. The growing of legumes, like alfalfa and sweet clover, tends to offset this loss, but it also tends to deplete the moisture supply for the following grain crop. Thus, yields following legumes are sometimes lower than they are following the previous grain crop and especially following fallow. No doubt, we shall see more nitrogen fertilizer used on these soils, but chemical nitrogen will not be enough. The organic matter must also be maintained through the proper use of crop residues.

Years ago, poor results from experiments with rock phosphate on Chernozems led some to conclude that they would not respond to phosphate fertilization. Chernozem soils are too rich in calcium and too high in pH for rock phosphate to become available. But later results indicate that strong feeders, like sweet clover and alfalfa, may show some responses even from rock phosphate. Greater increases, however, are obtained from the more soluble phosphates, especially for small grains and corn. This is partly because of depletion caused by long, continuous cropping, but more because of better varieties and better tillage practices that have made it possible for plants to use more phosphate efficiently. The use of phosphate is increasing and should increase a great deal more. The need, however, is likely to vary a good deal from one local type to another.

Potassium generally is not needed on Chernozems, although there are exceptions. It cannot be ruled out. Deficiencies of the minor nutrients are uncommon but may be expected here and there, if other fertilizers are used abundantly and especially with supplemental irrigation.

Desert soils

The soils of the desert are well supplied with mineral plant nutrients, except for the very sandy ones, but are low in organic matter and nitrogen. Often the mineral nutrients are poorly balanced, with excesses of some. Without irrigation, these soils are used only for extensive grazing, and moisture is the limiting factor for plant growth rather than fertility. Where irrigated, however, fertility problems become highly important. With technically sound irrigation and optimum moisture conditions, the ceiling set on nonirrigated soils by the moisture supply is lifted and other factors become limiting.

Of first importance is nitrogen. With legumes in the rotation, some nitrogen may be supplied by them, but ordinarily commercial fertilizer is also needed. Potassium is usually abundant in desert soils, but phosphorus is commonly low. Then too, deficiencies of the minor nutrients, like iron and zinc, are common. These conditions are partly stimulated by the high amounts of lime generally present. The fertility problems of irrigated soils are often further complicated by excesses of soluble salts, of salts in general, or of some specific toxic salt like borax.

Here is an important field where soil science, plant nutrition, and plant breeding come together. A good deal of active research is underway but more is needed. In view of the high yields that can be obtained with improvements in combinations of practices, we can expect the Desert soils to make significantly larger contributions to the world food supply.

Latosols *

Vast areas of potentially arable soils exist in the tropics, and here may rest a great deal of the future hope of mankind for abundant food; but the problems are great, and the research to guide us is little.

These red soils of the tropics, Latosols, are strongly weathered and highly leached. By standards used in temperate regions, they are low in all plant nutrients. Furthermore, they have low base-exchange capacities and high phosphate-fixing capacities.

The tropics, nevertheless, have several advantages. There is little or no frost. The growing season is as long as the moist season, say six, nine, or even twelve months. The limitations imposed by climate, therefore, are generally less severe than in the temperate and cold regions.

Already, we have indications of some peculiarities of management requirements and a hopeful glimpse here and there of production attainable under improved management.

Important in connection with fertility is the mineral cycle—the uptake of minerals and their return to the soil. Plants in the tropics grow rapidly and decay quickly. If an ion of calcium, let us say, were to circulate three times in the tropics to once in the temperate regions; the same ion would theoretically do three times as much work in one place as the other. Equal amounts of plant nutrients, therefore, might go farther in the tropics than in temperate regions.

Since the Latosols of the tropics have low base-exchange capacities and, hence, low nutrient-holding capacities, a continuous cover of plants

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* A term recently introduced to apply to a broad group of soils in the tropics that are red, leached, relatively high in iron and aluminum as compared with silica, relatively low in base-exchange capacity, and highly aggregated.

is almost a necessity. Under such cover, the tendency of rapid leaching of nutrients will be offset greatly by the equally rapid uptake of these nutrients by plants. Thus, the absorbed nutrients will again be brought to the surface and subsequently released upon the death and decomposition of the vegetative material. Generally, the common practice in the temperate regions of growing a single crop in a field, with periods without plant growth between harvest and sowing, is unsuited to the humid tropics. We need there to think of mixed cultures rather than monocultures, even rather than rotations of crops grown in monoculture.

Exposure of the soil to direct sunlight has been observed to be harmful to many tropical soils. Nearly continuous shade is essential for the maintenance of productivity. This is partly a matter of fertility and partly a matter of soil structure. The removal of all vegetation in some places even brings about severe drying at and near the surface, resulting in formation of a hard laterite crust or pan in soils such as Ground-Water Laterites.

The corridor system of crop rotation, perhaps best developed in the Belgian Congo, shows great promise as a good system for many tropical soils, especially where commercial fertilizers cannot be had economically. This system is simply a modification of the old practice of shifting cultivation. Under the corridor system, a given tract of arable land is divided into long strips, say 1,000 yards long and 100 to 300 yards wide. A corridor is provided for each year of cropping and for each year under forest fallow. These are arranged so that some stage of forest borders each corridor in crops. Besides, a few specimen trees may be left for partial shade and regeneration. On a comparatively good soil, one may have a six-year rotation of crops with twelve years of forest fallow. Such an area needs eighteen corridors. Each year an old corridor is returned to forest fallow and a new one cut and planted to crops (9).

Good results have been obtained from organic manures and composts in India (7) and elsewhere. Although the use of manures has increased yields in the temperate regions too, they are of special importance in the tropics because of the gradual release of nutrients in the organic matter as contrasted to mineral fertilizers. Where leaching

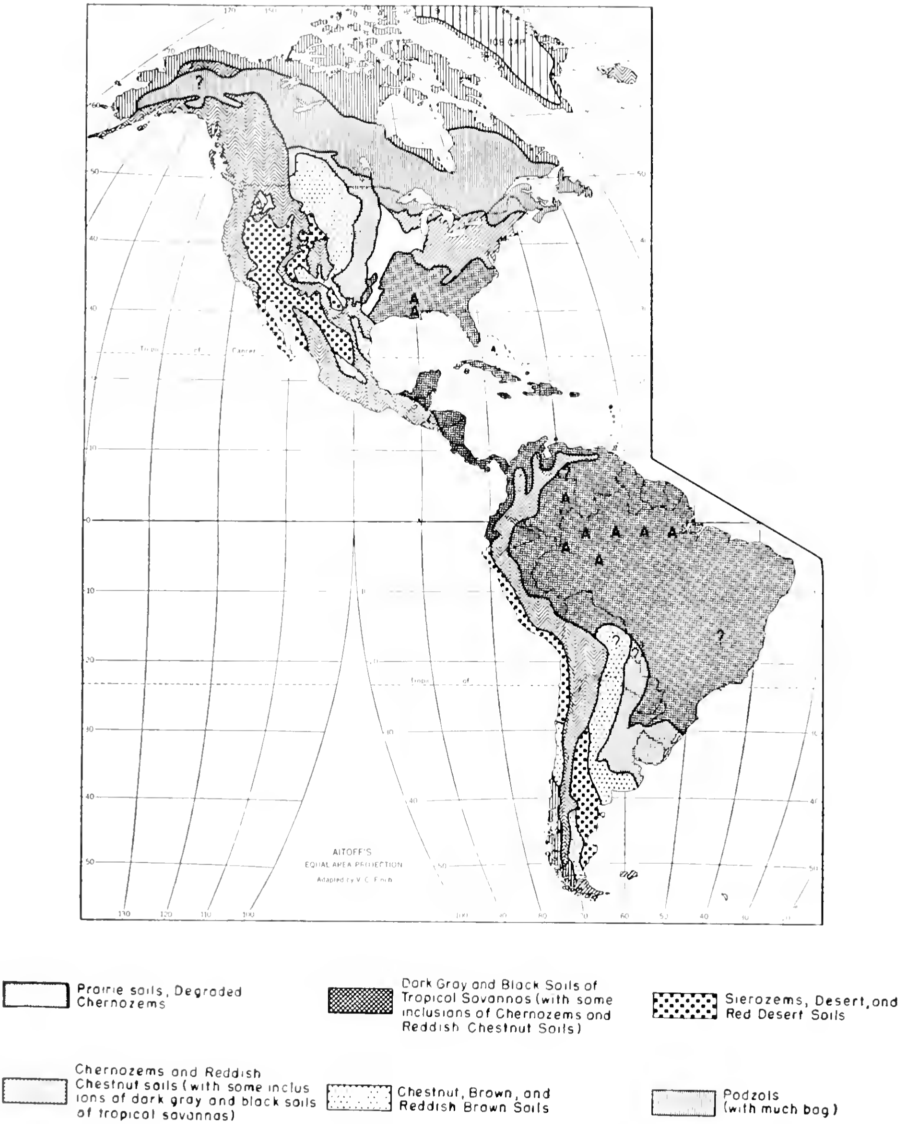
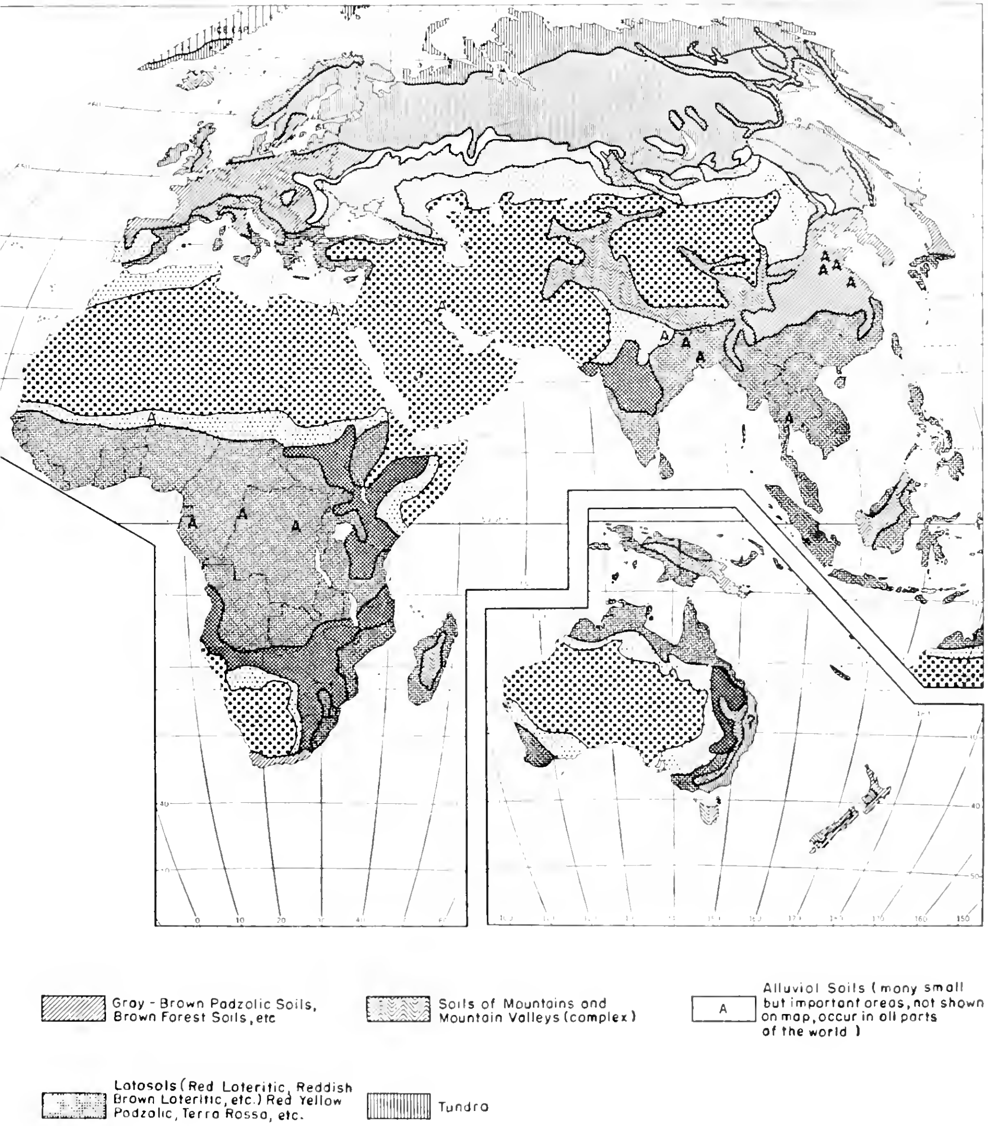


FIGURE 1. Provisional schematic soil map.


Gray - Brown Podzolic Soils, Brown Forest Soils, etc
Soils of Mountains and Mountain Valleys (complex)
A
Alluvial Soils (many small but important areas, not shown on map, occur in all parts of the world)
Latosols (Red Lateritic, Reddish Brown Lateritic, etc.) Red Yellow Podzolic, Terra Rossa, etc.
Tundra
40
40
50
0
10
20
150
160

is severe and exchange capacities low, this difference may be quite important.

Further, the balance among nutrients, important everywhere, is especially so in tropical soils. Much more research is needed to establish the correct fertilizer ratios to use on the many thousands of local soil types. The level of all nutrients is so low, in absolute terms, that an excess of one, caused by liming or fertilization, can easily upset the balance. Thus, in our ignorance, the use of compost has an advantage. Where a mixture of normal plants is used, one is bound to get a reasonably balanced supply of plant nutrients with decomposition. Someday we shall know from research the proper chemical mixtures to use. Yet, these mixtures can never substitute entirely for the good effects of mulch and the effects of organic matter in maintaining soil structure.

The large yields obtained with heavy fertilization on tropical soils in Hawaii, northern Queensland, and other places, where the products of industry are available in the tropics, give us a glimpse of enormous potentialities, provided agriculture and industry are developed together.

In the meantime, much can be done in the tropics to improve fertility and increase production without the use of mineral fertilizers. Our guess would be that scientific agriculture will increase yields even more in the tropics than it has on the Gray-Brown Podzolic soils of western Europe, and only a part of this will be due to fertilizers.

Besides the four major groups of soils discussed here, there are many others, each with unique fertility problems. But most of these soils are intergrades among the four broad groups.

SOIL DISTRIBUTION AND FERTILITY PROBLEMS

The provisional soil map shown in the accompanying figure gives a broad view of how the various kinds of fertility problems are distributed. This map is schematic and highly generalized; it is not detailed enough for local predictions of land-use potentialities.

Gray-Brown Podzolic soils and their associates are shown in the northeastern part of the United States, north-central Europe, and China. In relation to the total land area of the world, the extent of these soils is small. Yet, on them, both food production and fertilizer consumption are high, especially in Europe. In the prewar years, Europe alone used 59 per cent of all nitrogen, 63 per cent of all phosphoric acid, and 78 per cent of all potash in the world's fertilizer. Within Europe, these fertilizers were used largely in Germany, France, Denmark, Britain, and the Low Countries (*1*).

North of the Gray-Brown Podzolic soils is a great area of Podzols and their associates in North America and Eurasia. Large percentages of these are too stony, too sandy, too hilly, or too swampy for feasible cultivation. A large part of the area has only a very short growing season. However, some of the Podzols are under cultivation, and more could be cleared and cultivated. The fertility problems are similar in kind, but perhaps greater in intensity, to those of the Gray-Brown Podzolic soils. At least, lime and fertilizers are usually needed almost at once after clearing, for efficient production.

Between the Gray-Brown Podzolic soils and the Chernozems are the highly productive Prairie soils and Degraded Chernozems. They are less leached than the Podzolic soils but have more moisture than the Chernozems. The fertility problems are also intermediate. After some period of cultivation, the length of which varies with the local soil type, lime or fertilizers or both are required for efficient production on many of the local soil types. Phosphates are of first importance, generally, in mixed farming. Even then, supplemental nitrogen often gives responses on corn.

The Chernozem and Reddish Chestnut soils are found mainly in Eurasia, North America, and South America, although there are important areas in Australia and elsewhere. These are the great wheat-producing areas.

Besides the Chernozems, there are Dark Gray and Brown soils in the tropics. These are also fairly rich in mineral nutrients, more so than the Latosols, but they are relatively low in organic matter and nitrogen despite the dark color.

On the dry side of the Chernozems are the Chestnut, Brown, and Reddish-Brown soils. The fertility problems here are intermediate between those of Chernozems and those of Desert soils.

Vast areas of soils in deserts and semideserts are found in Africa, Asia, and Australia. Except for very extensive grazing, agricultural use is limited largely to the irrigated places. Although these are very important in the aggregate, they are too small to be shown on a map of this scale.

Finally, we emphasize the vast areas of Latosols, especially in Africa and South America. These are intensively used for crops in southeastern Asia and in nearby islands, in Hawaii, in parts of northern Queensland, and in many other small areas in Central America, South America, and Africa. Nevertheless, great regions are little developed. Even if crop production were increased on the Latosols only as much as it has on the Gray-Brown Podzolic soils during the past 150 years, the results would be enormous. The job is difficult; otherwise, it would not have waited so long.

LAND AND FERTILIZER POTENTIALS

It is obvious that any substantial increase in food production by boosting yields on old land, and by bringing in new, will depend upon vastly increased fertilizer use. What about potential fertilizer materials?

Two years ago, Salter (*6*) made some estimates along this line. These included not only 1,940,000,000 acres of existing crop land, but also the suggested 1,300,000,000 acres of new land.

Nitrogen can be obtained from the air wherever nitrogen-fixing plants can be built. Thus, for nitrogen, industrial facilities, not raw materials, are limiting. Known reserves of mineral phosphate and potash were estimated to be adequate for 2,000 and 500 years, respectively, within an assumed rate of phosphate use eight times the present one, and of potash use, eighteen times. These estimates, of course, take no notice of probable deposits in the many inadequately explored parts of the world. Nor do they allow for increasing efficiency in the use of fertilizer, which also can be expected. Although fertilizers are in short supply, the raw materials for them are abundant.

CONCLUSIONS

In our view, the soil problems, including the problems of fertility, are manageable in the biological and physical sense. Through the application of science and the expansion of research where needed, no predictable limit of production can be foreseen. Of course, there must be one, but it is very high.

This is not saying, however, that mankind will be well fed. The social, economic, and political problems are many and difficult. The technical problems of soils, plants, and animals, great as they are, are small by comparison.

Perhaps the really big question is: How badly do we want abundant food in the world? How much are we willing to sacrifice now, as individuals, as groups, and as nations? These are the sorts of questions that must be debated in terms of value judgments. They cannot be answered scientifically. What soil science says is that if people want an efficient agriculture, producing abundant food on a sustained basis, and are willing to develop the necessary social institutions, they may have it.

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PHYSICO-CHEMICAL AND BIOLOGICAL FACTORS AFFECTING NUTRIENT AVAILABILITY IN SOILS

CHAPTER

2

Soil as a Medium for Plant Growth

EMIL TRUOG

OUR BEST SOILS provide a well-nigh perfect medium for the growth of crop plants. Why should this be the case? Were soils specifically designed and created to support plants of the type we now have? More likely the correct answer is that plants by evolution gradually adapted themselves to grow on the soils as they happened to exist. This is substantiated by the belief that the first or primitive plants lived in water from whence one or more species migrated to the land, and there, by evolution, gradually developed into the many forms of land plants which today, as regards number of species, far surpass those of the ocean.

Whatever the answers may be to the questions just raised, the fact that nearly all our food and clothing and much of our housing come directly or indirectly from the soil provides ample reason and incentive for learning why some soils are good and many are poor media for crop growth, and how the poor soils may be made better and all good soils conserved indefinitely. Of the natural resources which are easily subject to serious deterioration and even complete destruction, soil is the most precious of all. To be sure, sunshine and water are just as important or even more so than soil, but fortunately neither is subject to destruction by the carelessness of man, although inland water resources may be greatly impaired through improper soil management practices.

The soil may, of course, be studied from several standpoints. The pedologist thinks of soils primarily in terms of their origin, form or morphology (profile characteristics), and classification. The soil physicist, the soil chemist, the soil microbiologist, and the soil conservationist each has his own field of special interest and study. A well-rounded soil scientist should have at least a working familiarity and knowledge in all of these special fields. On the other hand, many agronomists and horticulturists are interested in soil science solely from the standpoint of knowing how soils may serve as a medium for plant growth, and how they must be managed so as to produce satisfactory crops and at the same time be conserved for all time for this purpose. Although this paper is devoted primarily to matters of special interest to the agronomist, horticulturist, and plant physiologist, a brief discussion of soils from the pedologic viewpoint seems appropriate.

SOIL AS VIEWED BY THE PEDOLOGIST

The so-called science of pedology is the basic science of soils which deals with the origin, evolution, morphology, and classification of soils without any reference necessarily to their use for crop production. Soils are considered to be natural bodies or entities in themselves, deserving study as such, just as plants are to the botanist who often studies them without thinking of their economic value or use. This does not mean that the pedologist in pursuit of his vocation does not add to the economic welfare of society. Quite the contrary. By learning as much as possible about soils just as soils, a sound basic foundation is provided for their best use in the same way that the botanist provides this in the case of plants.

In order to determine the main characteristics of a soil so that it may be properly classified and evaluated, more than surface examination is needed; it is, in fact, necessary to expose the soil for observation and study from top to bottom. The pedologist calls this exposure a soil profile, which is simply a vertical cross-sectional view of a soil, extending from the surface to a depth, usually, of three or four feet, or to bedrock. It is the view one may see along a road cut, such as that pictured in Figure 1, where it will be noted in particular that the soil has three layers, called horizons by the pedologist.

It may well be asked—Why does a soil have layers or horizons which differ in characteristics? Why is it not uniform throughout? A consideration of how a soil forms and develops will suggest answers.

Let us suppose that an area of bare rock has become exposed to the action of air and water with accompanying ever-changing temperatures in a climate like that of Wisconsin. Under this action, cracks, fissures, and flaws develop in all rocks, into which seeds of plants carried by wind and birds may lodge, germinate, and grow. All of us have seen examples of this in a stone quarry, stone wall, or right in our rock garden, and have marveled at the ability of certain plants to anchor themselves in minute cracks and then flourish on virtually bare rock. Lichens and moss, which will grow on practically bare rock, are often the forerunners of the higher plants. The dense and compact growth of moss provides favorable conditions for the accumulation of both mineral and organic matter. Having gained a foothold, the plants help to hold the rock powder being formed in place against removal by wind and water, and in addition exert a powerful influence in accelerating the further disintegration of the rock. Once established, the plants start to shed and cast off leaves, roots, and other parts which become mixed with the rock powder and provide organic matter which serves as food for microorganisms brought in by wind, water, and other agencies. Thus the embryonic soil receives the touch of life which enables it to grow to maturity.

However, even after the beginning just described has been made, it may take 1,000 years to produce an inch of soil material. After a layer, usually eight to twelve inches deep, has developed, the further production of soil material by weathering of rock material produces a second layer which is quite different from the one above; it receives very little organic matter, being somewhat distant from the main source of such material, and its population of microorganisms is thus much lower; also, it is less subject to leaching and may, in fact, receive fine clay and other material from above. In time this layer becomes as thick as or thicker than the layer above, but, because of a lack of organic matter which gives all or a portion of the layer above a dark color, it usually has a yellowish, brownish, or mottled brownish to bluish color, depending upon the state of oxidation of the iron as determined by drainage and aeration.

Of course, the bedrock beneath keeps on disintegrating under the solvent action of water and the action of frost and other agencies. The weathering rock material exists first largely as a layer of loose gravel which receives additions continually from below as the bedrock disintegrates, but also loses material as its upper portion weathers more completely from gravel to soil and becomes incorporated as a part of the layer above.

Thus we see that in the development of soils from rock, it is natural and inevitable that the characteristics of the soil should vary from the surface downward. Although this variation is gradual, it is sufficiently marked in most cases to allow ready observation of three layers of the kind described. For convenience of reference as illustrated in Figure 1, the pedologist calls the upper layer the A horizon, the middle layer the B horizon, and the lower layer just above the bedrock the C horizon. Strictly speaking, the true soil, called the solum, consists of horizons A and B; horizon C is raw material out of which the soil proper is made.

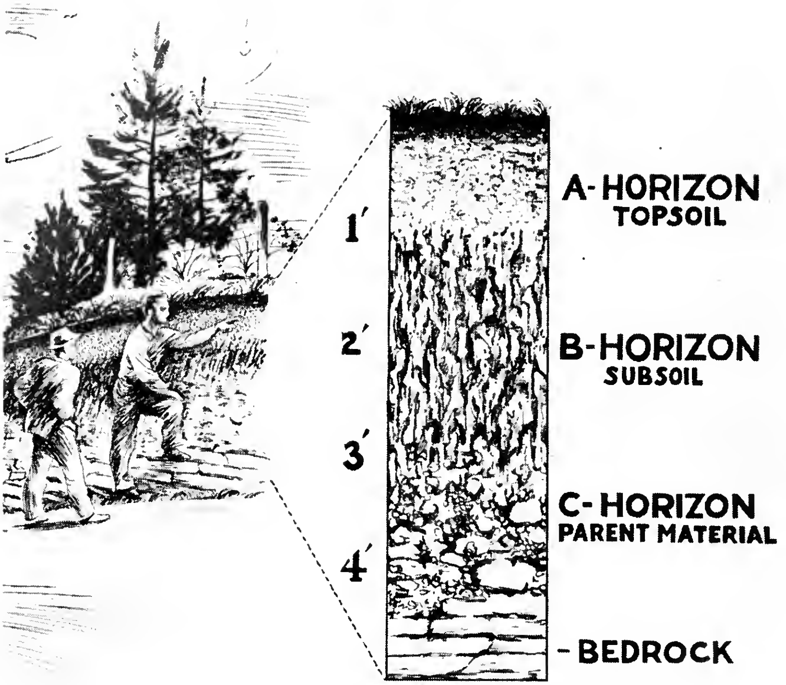

FIGURE 1. A vertical cross-sectional view of a soil showing the horizons (layers) that make up a soil. This is called a soil profile.

CHARACTER OF SOILS DETERMINED BY FIVE FACTORS

As is to be expected, great variations exist in the characteristics of the respective horizons of soils found in various places. These variations are due to the extent to which the following five main factors involved in soil formation have come into play. These five factors are climate, native vegetation, parent rock material, topography or slope, and age or maturity of the soil. The first two—climate and vegetation—are active factors and have to do with dynamic forces, such as weathering and leaching. The other three factors are of a passive nature and do not represent forces or activity in themselves, but they influence greatly the effects of the active factors on the product which remains and, hence, the character of a soil as found and its suitability as a medium for crop growth.

From a broad or world viewpoint, climate, which not only determines the rate of weathering and leaching but in turn also largely the kind of vegetation which grows, has the greatest influence on the general type of soils formed, particularly as regards the organic matter content and the color. It determines which of the so-called zonal soils will be formed, namely, podzol, chernozem, or laterite. However, within a local area, such as the northern half of Wisconsin which lies within a single climatic zone, parent rock material is the determining factor for the texture of the soils formed.

In a cool moist climate like that of northern Wisconsin where leaching is severe and coniferous trees predominate, the podzols or highly podzolized soils predominate. Whether of a sandy or heavier texture, they are characterized by an ash gray A_2 horizon and a brownish, yellowish, or yellow-blue mottled B horizon, which usually has a heavier texture than the A horizon. These soils, having been drastically leached, are strongly acid and low in available plant nutrients, particularly potassium. As media for crop growth, they are generally not satisfactory until limed and heavily fertilized with potash. The subsoil or B horizon of the heavier soils is often inadequately drained and aerated for many crops.

Compared with the northern part, the climate of the southern part of Wisconsin is quite different. Here, higher temperatures have caused more evaporation and transpiration and, hence, less leaching. Also, the higher temperatures have favored hardwoods rather than conifers, and this has further reduced leaching effects. As a result, the soils, being less leached, are less podzolized and, therefore, better media for crop growth. The fact that the parent rock material in the northern part of the state has been sandstone and igneous rocks and in the southern part largely limestone has also greatly favored the formation of better soils in the latter area.

If one goes south to humid semitropical or tropical regions, reddish or lateritic soils are quite generally encountered. These soils are usually low in organic matter and have generally suffered severe leaching. They are, however, generally well drained and aerated and when properly fertilized usually serve as excellent media for crop growth.

Going west from Wisconsin, one encounters regions of less and less precipitation. On reaching the subhumid region of the Dakotas where the annual precipitation averages 20 to 25 inches, it is found that tall grass vegetation has flourished instead of forest. Under these conditions, the chernozem and related soils were formed. They are rich in organic matter, have suffered little or no leaching, are rich in plant nutrients, and in many respects represent our best media for crop growth. The main failing is that the precipitation which accompanies them is not at all dependable. Thus we see that the climate which promotes the formation of the best soil media for crop growth usually does not have sufficient precipitation for highest crop yields.

To the west of the subhumid region of the Dakotas, one encounters the semiarid region where the average annual precipitation is less than 20 inches. Here, the limited soil moisture supply has restricted vegetation to the extent that the soils formed are practically devoid of organic matter. However, because of the virtual absence of leaching, these soils are usually well supplied with available nutrients and often make good media for crop growth when irrigated. Because of little or no leaching, these soils are generally alkaline, frequently to the extent of injury to crops. (See references *3*, *4*, *8*, and *9* for further details regarding origin and classification of soils and other pedologic matters.)

THE CONSTITUTION OF SOILS

Turning now from the brief consideration of the origin, gross morphology, and kinds of soils, there follows a discussion of the chemical, physical, and mineralogical constitution—that is, what might be called the anatomy and histology of soils. Matter exists in three physically distinct conditions or states commonly referred to as the solid, liquid, and gaseous phases. It is important to recognize that all three phases of matter, and in addition a "living phase" consisting of soil bacteria and other organisms, enter into the constitution of a soil when it is in proper condition to function as a medium for plant growth.

Thus, in the language of the physical chemist, soils are three-phase systems: there is the solid phase consisting of innumerable minerals and organic substances; the liquid phase consisting of the soil moisture or water in which relatively small amounts of the solid phase dissolve; and the gaseous phase, the soil air, which fills the pore space not occupied by the soil moisture. The total volume of the latter two in any soil must, of course, always equal the volume of the soil's pore space, although the volume of each fluctuates with the moisture content of the soil.

But a soil serving as a medium for plant growth is more than a three-phase system in the ordinary sense: it teems with microorganisms, and has, in addition, what may be called "a living phase," which must always be taken into account in any consideration of the soil as a medium for crop production. The constitution of the three conventional phases and also the "living phase" of the surface or plow layer of a typical silt loam soil is given in outline form in Table I.

The solid phase

It will be noted that the solid portion or phase of a silt loam occupies only about one-half of the volume of the soil, the remainder being, of course, pore space when the soil is free of moisture. The proportion of solid-to-pore space varies greatly among the different types of soils. In general, the heavier the soil—that is, the greater the content of finer material, especially clay, and also the greater the content of organic matter—the higher will be the percentage of pore space. Thus,

TABLE I

Constitution of the Soil (Plow Layer) of a Typical Cultivated Silt Loam at or near Optimum Moisture Content

I. Solid Phase: The soil proper, 50 per cent by volume

Inorganic portion, 95 per cent by weight		Organic portion, 5 per cent by weight
Coarser portion: Sand, silt, and coarse clay, particles $>0.2\mu$ diam., 80 per cent by weight. Largely primary minerals: Feldspars, micas, quartz, and many others	Fine clay or colloidal portion: Particles $<0.2\mu$ diam., 20 per cent by weight. Largely secondary minerals: Inorganic base-exchange material, iron oxides, silica, and kaolinite	Largely colloidal: Organic base-exchange material, lignin, cellulose, and proteins

II. Liquid Phase: The soil solution, 25 per cent by volume

Largely water solution of salts and gases. Concentration of salts usually ranges from 100 to 1000 p.p.m. of the dry soil. Calcium sulfate and bicarbonate often predominate. Sulfates, nitrates, chlorides, and bicarbonates of calcium, magnesium, potassium, and sodium present. Traces of many other constituents.

III. Gaseous Phase: The soil atmosphere, 25 per cent by volume

	Percentage by volume	
	In soil air	In ordinary air
Oxygen	20.0	21.00
Nitrogen	78.6	78.03
Argon	0.9	0.94
Carbon dioxide	0.5	0.03
	100.0	100.00

Both soil air and ordinary air may contain traces of hydrogen, ammonia, and oxides of sulfur and nitrogen. Figures for soil air represent a condition of good aeration and, for ordinary air, conditions some distance from cities.

IV. "Living Phase": The soil organisms, 5 tons of living tissue per acre

Bacteria	1,000,000,000 or more per gram of soil
Actinomyces	10,000,000 or more per gram of soil
Fungi	1,000,000 or more per gram of soil
Algae	100,000 or more per gram of soil
Protozoa	1,000,000 or more per gram of soil
Nematodes	10 or more per gram of soil
Earthworms	1,000,000 per acre in rich soils

Number of organisms varies greatly with season and kind of soil. Roots of a growing crop may equal or exceed in weight that of the soil organisms.

the pore space may approximate 33 per cent in sands, 50 per cent in silt loams, 60 per cent in clays, and 75 per cent or more in peats. The remainder, making up the total of 100 per cent, represents, of course, the solid phase in each case.

The solid phase is made up of inorganic and organic material. The proportion of each varies greatly in different soils. Well-drained upland mineral soils seldom contain more than 10 per cent of organic matter, and when they contain 5 per cent they are usually considered to be well supplied. Poor sands may contain less than 1 per cent; mucks contain 20 to 50 per cent; and peats contain over 50 per cent. Organic matter, which consists chiefly of plant residues in various stages of decomposition, plays a very important function in soils by favoring desirable physical, chemical, and biological processes. The maintenance of an adequate supply of organic matter in cultivated soils is of paramount importance and represents one of the major problems in practical soil management.

The inorganic portion consists of mineral particles varying in size from coarse sand grains, which can be seen easily with the naked eye, to clay particles which are too fine to be seen individually, even with the aid of a powerful microscope. The coarser particles consist largely of primary minerals, that is, minerals which formed or crystallized out when molten masses cooled to form the igneous (original) rocks. As found in soils, they represent the product of physical weathering solely: they have not been altered as regards composition by chemical weathering. For the most part, they usually consist of shiny crystals of quartz, mica, and feldspar. The finer particles consist almost entirely of secondary minerals—the new compounds which form and remain when primary minerals undergo chemical weathering. They consist largely of iron oxides, silica, and particularly the clay minerals which are tremendously important in soils because they have the property of holding plant nutrient cations in an exchangeable and readily available form. Details regarding the nature of these clay minerals and associated exchange phenomena are given in another paper of this symposium. Let it suffice to say at this point that the available plant nutrients are held largely in the finer clay particles and in the organic matter.

The liquid phase

The liquid phase of soils varies in volume, of course, with the moisture content of the soil. Immediately after a heavy rain the pores of the upper portion of a soil may be entirely or nearly filled with water. If the soil has adequate drainage, nearly one-half of this water is usually removed quite rapidly by the force of gravity. When a point is reached at which the attractive force of the soil for water equals the force of gravity, the removal of water by drainage ceases. At this point the pores of the soil may be about one-half filled with water; this water is not held in the pores as droplets, however, but as films on the surface of the soil particles. At this point, also, conditions are favorable for plant growth: aeration is adequate, the plant is able to draw on the water to supply its vast needs, and conditions are favorable for chemical and biological activity so that nutrients are brought into solution and thus made available for plant growth.

It will be noted in Table I that the concentration of soluble salts usually ranges from 100 to 1000 p.p.m. of the dry soil. Nutrient elements contained in these salts are, of course, available for the nourishment of growing plants. However, as will be seen later, plants are not dependent on just those nutrients which are dissolved in the soil solution. If the concentration of these dissolved salts reaches 2000 p.p.m. of the dry soil (10,000 p.p.m. of the soil solution in a soil containing 20 per cent of moisture), the salts may become toxic to many plants. Thus, it is seen that a proper regulation of the liquid phase as regards content of dissolved substances is a matter that requires much consideration and attention in practical agriculture.

Lack of adequate supplies of water probably limits crop growth more than any other factor. Because the subject of soil moisture is given special attention in papers that follow, it is not given further consideration here.

The gaseous phase

It has already been indicated that the gaseous phase of soils, that is, the soil air, varies inversely in amount with the amount of water present, and that when the pore space is no more than one-half filled with water, adequate aeration is provided. It will be noted in Table I that the composition of soil air as given is not markedly different from ordinary atmospheric air. The main difference is that the content of carbon dioxide may be 10 or 20 times greater in soil air. This is due to the rapid production of carbon dioxide in the respiration of soil organisms and plant roots, and its delayed escape to the air above because diffusion and other movement is greatly impeded in the fine pores of the soil. Under waterlogged conditions, the escape of carbon dioxide and entrance of fresh air almost ceases, and, thus, biological activity changes to an undesirable type in which toxic concentrations of methane, hydrogen sulfide, and other substances develop. Nearly everyone has noted how quickly an excess of water in soils, causing a lack of aeration, brings about the yellowing and death of plants. This emphasizes the great importance of a proper regulation of the gaseous phase of soils.

The "living phase"

When soils are in proper condition for crop production, they contain substantial amounts (often five tons or more per acre-plow-layer) of living matter in the form of bacteria, fungi, and other organisms listed in Table I. Matter animated with life attains special and unusual properties, and, because of this and its great importance in soils, it may well be given the special designation of the "living phase." Without this "living phase" of soils, plant residues would not decompose, and the resulting stagnation would in a relatively short time make crop production in the usual manner impossible. The role of this "living phase" in nutrient availability is discussed in detail in another paper.

THE pH OF SOILS

It is well known that the pH of systems which involve many chemical reactions and, particularly, biological activity and life is tremendously important. So without question, the most important single chemical characteristic of a soil as regards its suitability for plant growth is its reaction or pH status. Fortunately, this characteristic can be determined easily and accurately by means of simple tests that can be operated, if necessary, right in the field.

As is also true with many biological systems, the pH of soils involves both the solid and liquid phases. Since the two phases are either in or tending to attain equilibrium with each other as regards pH, it follows that any change in pH of either brought about by outside agencies is reflected in time in the pH of the other. Figure 2 illustrates this relationship.

In an acid soil, the acidity of the solid phase is due to the insoluble colloidal alumino-silicic or clay acids and humic acids, and that of the liquid phase largely to the presence of sulfuric, nitric, and hydrochloric acids as well as the ever-present carbonic acid, all formed for the most part through the activity of microorganisms. As these acids of the liquid phase are formed, there is a tendency for their hydrogen ions to take the place of the exchangeable bases of the solid phase. The higher the pH of the solid phase, the more complete the exchange. If the pH of the solid phase is near the neutral point, then the pH of the liquid

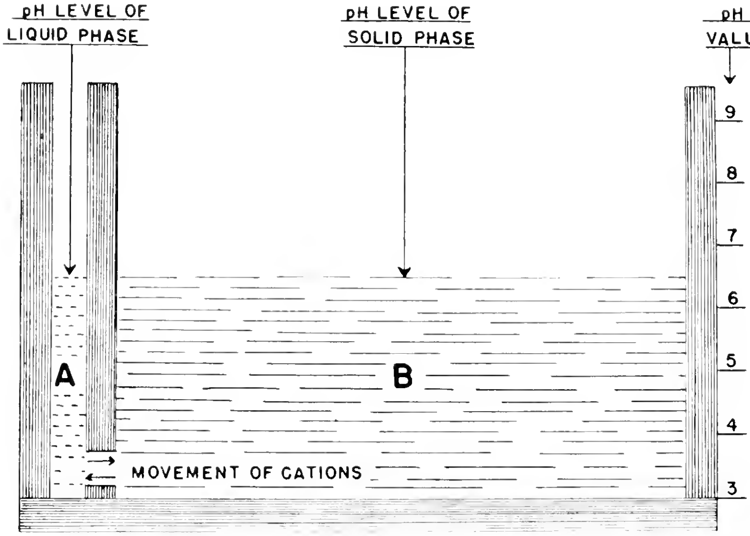

FIGURE 2. Schematic illustration of pH equilibrium relationship that exists between liquid and solid phases of a soil. Development of acids in A disturbs equilibrium and causes an exchange of H-ions by A for basic ions of B so as to readjust equilibrium. Addition of lime to a soil causes a reversal of the process. The tremendous cation capacity of B serves to buffer the low capacity of A against rapid changes in pH.

phase will likewise be maintained near the neutral point. Because the capacity of the solid phase of a good soil for exchangeable bases is very great, the solid phase is well buffered against any rapid drop in pH, and, since the liquid phase is in equilibrium with the solid phase, it also is well buffered. This is of tremendous importance because it prevents a rapid drop in pH of the liquid phase, such as would occur in nitrification.

When the pH of the solid phase becomes too low, the condition is commonly corrected by liming. As the lime dissolves, the pH of the liquid phase rises and there occurs an exchange of dissolved calcium for the exchangeable hydrogen of the solid phase. This continues until the pH of the two phases is equal or is in equilibrium.

The pH value of a soil, as commonly determined by means of the glass electrode placed in contact with a paste or thick water suspension of a soil, represents the pH at or near the surfaces of the colloidal particles. This is a composite value, representing both the liquid and solid phases.

Why is the pH of a soil so all-important? The pH is vital because it has a profound influence on many factors connected with the suitability of a soil for plant growth. An outline statement of this influence follows.

I. The direct influence
    1. A toxic or destructive effect of H and OH ions on root tissues
    2. An unfavorable effect of low pH on the balance between the basic and acidic soil constituents available for absorption by plants
II. The indirect influence due to effect on
    1. Availability of nutrient elements
    2. Activity of soil microorganisms
    3. Toxicity of various substances due to high solubility
    4. Physical condition of the soil
    5. Prevalence of plant diseases
    6. Competitive powers of different species of plants

The direct influence

Soil acidity or alkalinity may exert an unfavorable influence on crop growth in two direct ways. There is first the possible toxic or destructive effect of acidity or alkalinity on the root tissues. When plants are grown under controlled conditions at different pH values as regards the nutrient medium, it is found that a destructive action of the root tissues by acidity or alkalinity usually does not take place until the pH drops below 4 or rises above 9, respectively. It has been found that cereals are considerably more resistant to low pH than alfalfa. (See references 5, *6*, and *10* for details of experiments in which these crops were grown at varying pH levels.)

Since very few soils become more acid than pH 4.5, it seems logical to conclude that the injurious influence of soil acidity on plant growth is seldom due to a direct toxic or destructive action on the root tissues. This is further supported by the fact that the natural reaction of the root sap of most agricultural plants falls in the same pH range, that is, 6 to 4, as that of many acid soils. It is to be expected that plant roots will withstand an external acidity which is of the same order as their natural internal acidity.

The second and much more important direct effect of soil acidity on certain plants is the unfavorable balance or excess which it creates of acids over basic constituents which are available in the soil for absorption by the plants. In order that the metabolic processes of a particular species of plant may proceed satisfactorily, it would seem to be necessary that a certain balance exist between the acidic and basic nutrients (anions and cations other than hydrogen) which are available for absorption. This contention is supported by findings which show that each species of plant when grown under different conditions strives to maintain a rather constant total equivalent base content. A deficiency of one base, for example, calcium, is compensated for by the absorption of an equivalent amount of other bases, such as magnesium and potassium. Thus, in a distinctly acid soil, some of the nitrate and sulfate, when present, exist in the soil solution as free nitric and sulfuric acids, respectively, and are absorbed as such by plants growing thereon; moreover, the more acidic the soil, the greater the proportion absorbed as free acids, and the more difficult it becomes for plants to counteract all this by the absorption of exchangeable calcium and other bases in the form of the bicarbonate. On the other hand, when the soil reaction is at about pH 6.5 or higher, practically all the nitrate and sulfate exist as neutral salts, particularly calcium and magnesium salts, and will be absorbed as such by a growing plant; in addition, ample amounts of calcium bicarbonate for absorption and use will be formed through the action of carbonic acid on exchangeable calcium.

It is well known that plants vary greatly in their preference and tolerance as regards the pH of the soil or other medium in which they are growing. For example, the most favorable pH range of alfalfa in this respect is considerably higher than that of timothy. If the contention made in the preceding paragraph is valid, then, chemical analyses should reveal a higher proportion of basic to acidic constituents in the alfalfa than in the timothy. Accordingly, in Table II, the results of such analyses for these two crops are presented. The percentages of constituents present have been recalculated and presented in terms of milliequivalents of cations and anions, respectively, so that the total amounts of basic and acidic constituents present may be compared on a chemical equivalent basis. This is necessary if the comparison is to be valid for the purpose at hand. For example, the basic oxides Al_2O_3 and CaO are expressed as milliequivalents of the cations Al^{+++} and Ca^{++}, and the acidic oxide P_2O_5 and N as milliequivalents of the anions PO_4^{---} and NO_3^-.

It will be noted that on a total milliequivalent basis, alfalfa contains somewhat more acidic than basic constituents. However, it is ordinarily expected that through the medium of symbiotic nitrogen fixation, alfalfa should obtain at least two-thirds of its needed nitrogen from the air. Nitrogen thus obtained brings no bases with it into the plant and, hence, a subtraction representing two-thirds of this nitrogen is made from the total of acidic constituents. After this is done, it will be noted that the alfalfa plant must get from the soil nearly twice as much of the basic as of the acidic soil constituents. Calculations indicated in the table show that on the basis of these data, 41.6 per cent of the basic elements, particularly calcium, magnesium, and potassium, must be absorbed as the carbonate or bicarbonate, which, because of the weakness of carbonic acid, provide the same end result as though these elements were absorbed as free bases. The possibility of absorbing these elements in adequate amounts as the carbonates is dependent on a high degree of base saturation (low acidity) of the clay and humic acids.

TABLE II

The Amounts of Basic and Acidic Constituents in Alfalfa and Timothy Expressed both as Percentages and Milliequivalents per 100 Grams of Oven-dry Tissue

Base-forming Constituents			Acid-forming Constituents		
Constituent	Per cent	Milliequivalents as cations per 100 g.	Constituent	Per cent	Milliequivalents as anions per 100 g.
			Alfalfa*		
Al_2O_3	0.054	3.18	SiO_2	0.070	2.33
Fe_2O_3	0.020	0.75	Cl	0.100	2.82
CaO	2.970	105.92	P_2O_5	0.638	26.96
MgO	0.578	28.67	SO_3	0.527	15.36
K_2O	2.146	45.56	N	2.674	191.00
Na_2O	0.208	6.71			
MnO	0.004	0.11			238.47
			Less 2/3 of N assumed taken from air		127.33
Total		190.90	Total taken from soil		111.14
$\frac{190.90 - 111.14}{190.90}$ = 0.416; therefore, 41.6% of bases need to be absorbed as carbonate and bicarbonate.					
			Timothy†		
Al_2O_3	0.053	3.12	SiO_2	1.851	61.64
Fe_2O_3	0.155	5.82	Cl	0.583	16.44
CaO	0.332	11.84	P_2O_5	0.474	20.03
MgO	0.220	10.91	SO_3	0.460	11.50
K_2O	2.190	46.50	N	0.990	70.71
Na_2O	0.017	0.55			
MnO	0.006	0.11			
Total		78.85	Total		180.32
$\frac{180.32 - 78.85}{180.32}$ = 0.563; therefore, 56.3% of acidic constituents may be absorbed as free acid and none of basic constituents need to be absorbed as carbonates and bicarbonates.					

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*Data for alfalfa are based on analyses by O. C. Magistad of five Wisconsin samples cut at hay stage.

†The figure for nitrogen content of timothy was taken from Henry and Morrison, "Feeds and Feeding." All other data for timothy are based on analyses of three field samples cut in bloom stage and reported in U.S.D.A. Bur. Soils Bul. 600 (1917) p. 11.

An examination of the analytical data for timothy reveals a much different situation in this connection. This plant (on a milliequivalent basis) requires more than double the amount of the acidic than of the basic constituents. There may be some question as to the propriety of including silica (SiO_2) and chlorine in these calculations because of the weakness of the former as an acid and nonessentiality of both as plant constituents. However, even when these two constituents are disregarded in the calculations, a considerably greater equivalent amount of acidic than of basic constituents is still required by timothy. Thus, the timothy plant can absorb a considerable portion of its nitrogen and sulfur needs in the form of free nitric and sulfuric acids without upsetting the internal nutrient balance of needed acids and bases.

In the above considerations it is assumed that all the nitrogen derived from the soil is absorbed as the nitrate. Absorption by plants of some of the nitrogen in the ammonium form as a cation will not alter materially the deductions made, since alfalfa will still have a much greater base requirement than timothy.

It has been noted that alfalfa often grows much more satisfactorily on distinctly acid soils if these soils happen to be well supplied with available nitrogen. However, in the case of nonacid and very slightly acid soils, the supply of available soil nitrogen is not crucial providing the alfalfa is properly inoculated. The foregoing discussion accords with these observations. When the alfalfa has access to soil nitrogen, much of the nitrogen absorbed carries a base with it even though the soil is acid. However, if the plant is dependent upon atmospheric nitrogen, then no bases are carried in with the nitrogen, and the plant must compensate for this by absorbing from the soil considerable amounts of the required bases in the form of the carbonate or bicarbonate, such as calcium bicarbonate. The carbonates and bicarbonates, because of the weakness of the acid radical, function in the plant much like a free base. Only when soils are not very acid can they be obtained in adequate amounts for plants like alfalfa which have a high requirement.

The indirect influence

The indirect influence of soil reaction on plant growth may be exerted in a number of ways. Of tremendous importance is the influence due to its effects on the availability of the plant nutrients. Strong acidity is, in general, unfavorable, while very slight acidity (about pH 6.5) is favorable. Figure 3 presents the subject diagramatically. It will be noted that at strong acidity, there is a marked drop in the supplies of available nutrients, which may exist as exchangeable cations. This is, of course, to be expected, because with increasing acidity, increasing amounts of these nutrient elements which might exist as exchangeable cations become replaced by hydrogen. In certain ranges on the alkaline side, some of the nutrient elements become much less soluble and available. A full discussion of this subject is beyond the scope of this paper and may be found elsewhere (*11, 12*).

The indirect influence of soil reaction on plant growth because of its effects on the activity of soil microorganisms is extremely important, particularly as related to the availability and fixation of nitrogen. In general, the more desirable types of biological activity in soils are promoted by a soil reaction that is neutral or nearly so.

Increasing acidity favors the solubility of aluminum and manganese, and also copper and zinc and other heavy metals should they happen to be present in undue amounts. When soil acidity becomes more intense than pH 5.0, concentrations of soluble aluminum and manganese which are toxic to certain plants may occur in some soils. Unless built up through the extensive use of spray materials or the addition in other ways, toxic concentrations of copper and other heavy metals seldom if ever occur.

The physical condition of heavy soils, especially, is generally known to be affected unfavorably by strong acidity which gives rise to a calcium bicarbonate supply in the soil solution which is insufficient to keep the clay well flocculated—a condition that is necessary for full promotion of the desirable granular or crumb structure. In sandy soils, a high level of available lime (low acidity or absence of it) may help to counteract excessive looseness by acting as a binding agent. Improvement of the physical condition of soils in the ways indicated helps to promote a satisfactory regulation of the air and moisture supply of soils; this, in turn, promotes the more desirable types of biological activity and chemical reactions. A highly acid or alkaline condition, by inducing deflocculation, causes a movement of colloidal material from the surface soil

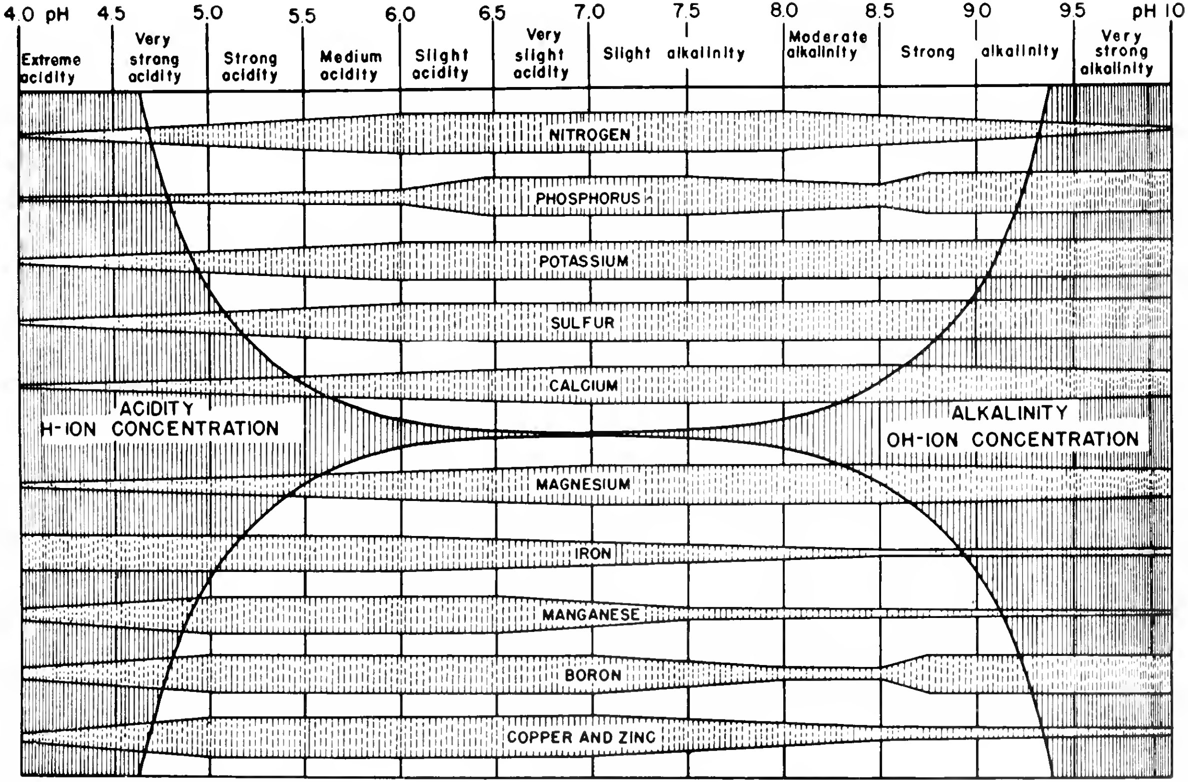

FIGURE 3. Chart showing general trend of relation of a soil's reaction (pH) and associated factors to the availability of its plant nutrient elements. Each element is represented by a band whose width at any particular pH value indicates the relative favorableness of this pH value and associated factors to the presence of the element in readily available forms (the wider the band the more favorable the influence), but not to the actual amount necessarily present, this being influenced by other factors, such as cropping and fertilization.

into the subsoil where it may be precipitated as a hardpan. The depletion of the surface soil of its colloidal material and the formation of a hardpan have an unfavorable influence on soil fertility.

Some soil-borne plant diseases are favored by an acid condition, while others are not. Certain fungous diseases like "finger-and-toe" develop to a harmful extent only in acid soils, whereas other plant diseases like the potato scab are most serious in well-limed soils, and, hence, some acidity (pH 5.2) is desirable under many conditions for potato growing. Aside from the cases known to have a direct relationship to plant diseases, there are a few special plants like the cranberry, blackberry, and watermelon for which a soil of at least slight acidity seems desirable and in some cases necessary for the best growth. It is not known just why these plants grow better on an acid soil, but it seems possible that in some cases plant diseases or malnutrition due to a lack of iron where the pH is too high may again be factors.

The influence of soil reaction on the competitive powers of different species of plants to establish themselves and crowd out others needs to be recognized. For example, the common sorrel (*Rumex acetosella*) is sometimes said to grow best on acid soils. However, when grown under controlled conditions free of competition by other species of plants, it grows better on neutral than acid soils. It is commonly found growing on strongly acid soils because there it usually meets with less competition from other weeds as well as crops. It would be found growing even better on the less acid soils were it not that here other plants grow so well and vigorously that they crowd out the sorrel.

Undoubtedly, competition working in conjunction with soil acidity or alkalinity in the way just indicated has a marked influence on the powers of different species of plants for establishing and maintaining themselves under natural conditions, and thus has affected the character of the native vegetation found in many regions.

Crop plants as well as plants in general vary greatly as regards the reaction of the nutrient medium in which they grow best. Some species of plants grow best in an acid medium, some in an alkaline medium, and others in a neutral or nearly neutral medium. The reasons for these reaction preferences are due to the direct and indirect influences of soil reaction just discussed.

SOILS ARE FRUGAL CUSTODIANS OF PLANT NUTRIENTS

The question is frequently asked, Is there not great danger of loss of plant nutrients by leaching when fertilizers are added to soils? Or, What about fixation of the nutrient elements by the soil so that plants cannot use them? The answer is that good soils are frugal custodians of the plant nutrients naturally present in or added to soils. If that were not the case, soils would rapidly become so depleted of fertility elements by careless and exhaustive cropping, that a protective covering of crop plants, or even weeds, would not be produced. Under these conditions, erosion would be terrific and disastrous.

The three availability categories of the nutrient elements

Just how does a good soil perform the miracle of supplying plants with adequate amounts of nutrient elements and still prevent undue loss by leaching? It does this by forming combinations of the nutrient elements of varying degrees of solubility or availability. For convenience of exposition and consideration, it may be said that nutrient elements are held or stored in soils in three degrees or categories of availability—namely, (*a*) readily available, (*b*) moderately available, and (*c*) slowly or difficultly available.

To produce a good crop, it is necessary that the supply of readily available forms of nutrients be high enough so that much or most of what is needed by any crop can be obtained directly from these forms. However, it is a great mistake to assume that plants get no nutrients at all from the less available forms. Failure by some to recognize that plants obtain some share of their requirements from nutrient forms of all degrees of availability has caused much confusion in their thinking about this matter. Also, it should be recognized that there is a continual transformation of each nutrient element from forms of one degree of availability to forms of other degrees of availability, and that the main direction of this transformation is determined largely by the relative proportions of the various forms present.

Figure 4 provides a schematic representation of the three availability categories of plant nutrients in soils. Transformation from one category to the others is represented by arrows in the connections between the categories, and the relative rate of possible transformation is indicated roughly by the width of the connections.

Forms of potassium in relation to availability categories

To serve as an example, the forms of potassium which make up the various categories of this element are given in the diagram. In this case, the exchangeable form makes up nearly all of the readily available category. Only small and rather insignificant amounts exist as water-soluble salts, such as nitrates and sulfates.

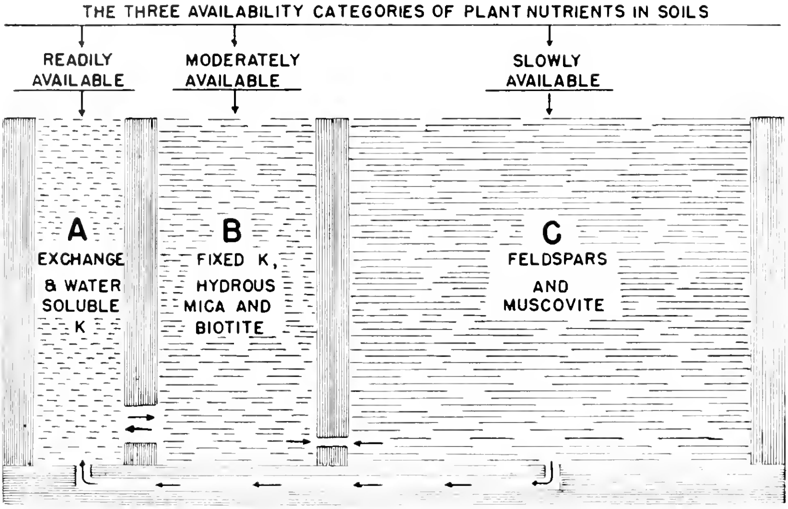

FIGURE 4. Schematic illustration of availability categories of plant nutrients in soils (exemplified with the element potassium) and transformation from one category to another. Arrows indicate the direction of transformations, and the wider the connection the more rapid the rate of transformation.

Moderately available potassium (extracted with dilute acid) exists in the so-called "fixed state" as hydrous mica (illite) and biotite. It appears that at high levels of exchangeable potassium, some of this potassium gradually becomes transformed to a nonexchangeable or fixed form. Possibly in this transformation the montmorillonite which holds the exchangeable potassium goes over to hydrous mica or some similar type of mineral. Since biotite contains ferrous iron which suffers oxidation on exposure to air and water, this mineral is quite readily subject to breakdown and release of its potassium.

The potash feldspars and muscovite contain practically all of the slowly or difficultly available potassium. Muscovite, unlike biotite, contains no ferrous iron and, as a result, is tremendously resistant to weathering and release of its potassium. Potash feldspars, being dense crystalline silicates, weather slowly. This is particularly true of microcline. Although soils frequently contain 30,000 to 40,000 pounds per acre-plow-layer of muscovite and feldspar potassium, usually not more than five to ten pounds of this become available annually for crop use. However, if little or no plant growth is removed from land for several years and leaching is not too severe, there results a sufficient accumulation from this source to be a potent factor in supplying potassium to one or more subsequent crops. The so-called "resting of land" owes its virtues to processes of this kind.

Let us now suppose a crop of corn is growing on the soil in question. What happens? There is a heavy drain on category A and, hence, its pressure for transformation to category B drops. As a consequence, the rate of transformation from B to A exceeds the reverse transformation and there is a gradual replenishment of A from B. After the growth of a heavy crop, it may take several months or more for the replenishment to run its course so that A and B are again in equilibrium. That is why the amount of readily available potassium found in a soil by test in the fall after the removal of a heavy crop is sometimes considerably lower than the amount found the next spring.

It will be noted in the diagram that there is a narrow connection between categories A and C, and the arrows indicate some transformation from C to A. However, the rate of this transformation is so slow that what may be transformed during a growing season is sufficient to supply only 5 to 10 per cent of crop needs. Thus it is apparent why the amount of potassium in A must be kept up to a certain level if a crop is to be adequately supplied. In practice it has been found that category A should contain about 200 pounds per acre-plow-layer of potassium to assure good yields of corn, alfalfa, and other general farm crops. Potatoes, sugar beets, and truck and garden crops require a level which is two to three times as high. Fortunately, the level of potassium in category A is easily determined by means of a chemical test.

The amount of potassium in category B may be several times that in category A, but ordinarily during a growing season crops probably get no more than 10 to 20 per cent of their potassium needs from the former. The very important consideration in this connection is that during the time when crops are not growing, there occurs a flow of potassium from category B to A to make up some of what the previous crop has removed from A. Transformation of potassium from category B to C and the reverse of C to B is extremely slow. In fact, there is some question whether or not these transformations in the case of potassium take place at all, especially from B to C.

Now, what happens when soluble fertilizer potassium is added to a soil in which categories A and B are at equilibrium? At first, most of this potassium rapidly goes into the exchange form of category A. This causes a disturbance of the previously established equilibrium between A and B, and as a result there occurs a flow of potassium from A to B until equilibrium is again established. That is why it takes more than the calculated amount of potash fertilizer to raise the level of potassium in A to some prescribed point. However, it is important to recognize that this potassium which is transformed to category B is not lost. It is simply stored more safely against leaching and reckless use by careless cropping. Yes, the soil is a frugal custodian.

What is the situation in virgin soils as regards levels of potassium in the three availability categories? Let us consider first the conditions under which the podzols are formed. Here, leaching is so severe that even a frugal soil is unable to stem the tide of relatively rapid loss of potassium from category A. This causes a continual flow from B to A, and, as a result, the level of potassium in categories A and B becomes low. The flow of potassium from C to A is so slow that virgin podzols in northern Wisconsin, containing less than 100 pounds per acre-plow-layer of category A potassium, have on the same basis 30,000 to 40,000 of this element in category C. This is further evidence that category C cannot be depended upon to furnish much of the current needs for profitable cropping other than forestry where the annual drain is low and only one crop may be removed in 50 years. Moreover, during this time, the dropping of needles and leaves provides a continual return of nutrients to the soil. Even with this return, podzols in general are so low in category A and B potassium that soon after being brought under cultivation they require heavy applications of potash fertilizer.

Going now to the slightly podzolized soils of southern Wisconsin, it is found that the supplies of potassium in categories both A and B are usually five to ten times as high as in podzols to the north, although the supplies in category C may be quite similar. This further emphasizes the fact that transformation from category C to A is very slow. Because of the much greater supplies of category A and B potassium in the less podzolized soils, it is found that many of them may be cropped for 50 years or more without need of potash fertilization.

The greatest supplies of category A and B potassium are found in the chernozems and other alkaline soils formed under limited rainfall. Here the build-up may be high enough to last for 100 years or more of cropping without the need of adding commercial potash fertilizer. (A more complete discussion of potassium in this connection may be found in references *1*, *2*, and *7*.)

Thus far, nothing has been said about phosphorus, nitrogen, calcium, and other nutrient elements in relation to availability categories. In general, they follow much the same pattern as potassium, but, of course, the types of compounds involved may be quite different.

Forms of calcium and magnesium in relation to availability categories

Calcium and magnesium as regards category A are very similar to potassium, also being held there as exchangeable cations. In fact, unless the soil is quite acid or the pH is higher than 9, calcium and magnesium practically always make up the great bulk of the total exchangeable cation content of a soil; and, if the soil is quite acid, then calcium and magnesium are usually supplied in the form of lime to bring up the supply of these elements in category A to the desired level so as to attain a pH of at least 6.5.

The calcium and magnesium of category A do not become fixed like potassium and revert to category B. In fact, relatively little category B calcium ordinarily exists, unless the exchangeable calcium still remaining in distinctly acid soils be thus designated because of its rather low availability compared with much of that existing at pH 6.5. Magnesium like potassium tends to form secondary silicates to a much greater extent than calcium, and, hence, its greater tendency to go into forms which fall in category B.

Because calcium feldspars and other calcium silicates and most of the primary magnesium silicates weather relatively rapidly, the supply of calcium and magnesium in category C is generally very much lower than potassium, especially in soils of the humid regions. That is one reason why in these regions it is generally necessary, from time to time, to add large amounts of calcium and considerable magnesium in the form of lime.

Availability categories of phosphorus, nitrogen, and sulfur

Since phosphorus exists in soils largely as the anion PO_4^{---}, it cannot exist in category A in the same form as most of the calcium, magnesium, and potassium. It can, nevertheless, probably exist attached to aluminum and iron hydroxides and silicates as an exchangeable anion, and a small portion of this may possibly be considered as belonging to category A. However, the main bulk of category A phosphorus in soils well supplied in this respect exists as calcium phosphate. Experience teaches that for good yields in general farming, a soil should contain at least 50 pounds per acre-plow-layer of category A phosphorus. For truck and garden crops, double and treble this amount is required for best results.

The forms of phosphorus which make up categories B and C have not as yet become well defined. Possibly, much of the phosphorus attached to aluminum hydroxide and the silicates can be considered as belonging to category B, and most of that attached to iron hydroxide and all existing as apatite as belonging to category C. Details regarding the manner in which phosphates and other nutrient anions are held and become available in soils are given in another paper of this symposium.

Because nearly all of the nitrogen of soils is generally associated with organic matter rather than mineral matter, it might be expected that the pattern of availability categories for nitrogen would be radically different from what it is for, say, potassium. This, however, is not the case. In order for the soil to be a frugal custodian, it appears necessary or desirable that the same general pattern be followed for all of the nutrient elements.

The nitrogen of category A usually exists largely as fresh crop residues and recently applied manure and nitrogen fertilizers. Very small amounts may be held in exchangeable form as NH_4^-.

When organic matter accumulates for long periods as it has in chernozems, and mucks and peats, true humus is formed, and much of the nitrogen contained therein, possibly two-thirds or more, is of low availability and belongs to category C; the remainder falls in category B and largely provides the nitrogen for crops when these soils are brought under cultivation. Under continuous cropping, very little of the nitrogen added in the form of crop residues, manure, and fertilizer becomes transformed into category B or category C. However, when sod crops occupy the land continuously for several years, such transformation does take place to an appreciable extent. Thus, it is apparent that categories B and C with respect to supplies of nitrogen can only be built up in practical farming by keeping the soil in sod crops a good share of the time.

Sulfur, as regards availability categories, follows a pattern quite similar to nitrogen when it is present as a constituent of organic matter. It also exists in mineral forms which fall into all three categories, but often to the greatest extent in category C.

Availability categories of the minor nutrient elements

The four minor nutrient elements—iron, manganese, copper, and zinc—which exist in soils primarily as cations follow much the same pattern as calcium and potassium. Contrary to this pattern, iron and manganese particularly when the pH is above 6.5, readily form the insoluble higher oxides which belong to categories B and C. Boron follows a pattern quite similar to nitrogen except that it changes rapidly to category C at moderate alkalinity, and again rapidly to A and B at high alkalinity.

To be sure, many if not most of the details of the matter just discussed are still to be worked out, especially with respect to the minor elements. However, enough is known to make it safe to repeat that a good soil is, indeed, a frugal custodian of all the nutrient elements.

SOILS NEED ADEQUATE CAPITAL

It is generally recognized that the success and survival of a business venture depends primarily upon adequate working capital and good management. In a sense, the same situation prevails in the case of a soil serving as a medium for plant growth. Here, the supply of readily available nutrient elements may be likened to working capital; the moderately available, to negotiable bonds and securities; and the slowly available, to fixed assets or investments in real estate. The farmer must, of course, provide the management.

If a soil, located favorably as regards climate and surface drainage, is provided with an adequate supply of the essential plant nutrients in readily available forms, it is really astounding how satisfactorily crops will usually grow and how well unfavorable physical conditions of the soil will in time become corrected. In this connection the writer has in mind a specific case. In North Central Wisconsin there exists a large area of podzolic soil called Spencer silt loam. This soil is strongly acid throughout the profile, and is therefore low in its supply of available bases, particularly potassium. Also, the subsoil or B horizon is very compact and rather impervious to both water and roots. It was generally surmised that successful alfalfa culture on this soil would never be possible, even if adequately limed and fertilized, because of the impervious and poorly drained subsoil.

About twenty years ago a farmer by the name of James Asplin acquired some typical Spencer silt loam. Apparently sensing that the soil lacked a lot of something, Mr. Asplin proceeded to apply about twenty-five wagon loads (25 tons) per acre of wood ashes to his soil. The ashes were obtained from a nearby sawmill and probably contained about three per cent of potassium, 50 per cent of lime, and appreciable amounts of phosphorus and other mineral nutrients. Thus, approximately 1500 pounds per acre of potassium in the form of the carbonate and an abundant supply of readily available lime and other mineral nutrients were provided. In other words, the soil was provided with adequate working capital.

Following the application of the ashes, crops began to grow with great vigor. Even alfalfa became a successful crop on a soil whose highly acid, impervious, and poorly drained subsoil would seem to rule out this crop for all time. Just lately, an alfalfa plant, which among many others has persisted for about fifteen years in a subsequently unplowed corner of one of the fields treated with ashes, was carefully dug up so its root development might be examined.

The root system of this plant was found to be both deep and extensive. Large tap-roots had gone to a depth of over four feet. Gnarled knots found on the roots indicated clearly that at certain stages the growing points of some of the roots probed about persistently in search for a tiny opening which would allow further downward penetration. Undoubtedly, the vigor in the plant needed for this relentless probing and development was engendered by the liberal supply of plant nutrients.

Extensive field tests in recent years show conclusively that with adequate liming and fertilizing, Spencer silt loam becomes a very good medium for the growing of alfalfa and many other crops. Under this treatment and a rotation which includes a tap-rooted legume like alfalfa, there is little question that in time this soil can be vastly improved. When these large tap roots die and decompose, the subsoil will be provided not only with organic matter, but also, and this is probably even more important, with large pores for aeration and drainage.

Of course, there are some soils which, because of shallowness, high water table, or other physical defects, cannot be made productive by applying lime and fertilizer. Then there are the saline soils—soils which because of restricted leaching contain such an abundance of salts (some of these may be actual nutrients) that a toxic condition for plant growth is created. To carry out the simile used previously, it may be said that such soils are over capitalized and, for amelioration of this condition, require special treatment.

Wherever soils have suffered severe leaching, they usually lack an adequate supply of plant nutrients for good crop production. However, when this lack is corrected, the favorable results are often phenomenal. The present high productivity of podzolic soils in the Scandinavian countries furnishes proof of this.

DETERMINING A SOIL'S FERTILIZER NEEDS AND FITNESS FOR PLANT GROWTH

If a soil has been properly classified much will be known with respect to its management needs and crop adaptability. If it is a podzol, then we know it will be acid and low in available supplies of most of the plant nutrients. Soon after being brought under cultivation, it will need lime and fertilizer, particularly potash, if legumes like clover and alfalfa are to be grown. If it is a fine-textured podzol, then we also know that the subsoil will probably be rather tight and impervious. This may require special treatment.

On the other hand, if the soil is a chernozem, that will immediately stamp it as being well supplied with nutrients and an excellent medium for plant growth. It will need no lime and probably no fertilizer for many years. The first fertilizer needed will probably be phosphate. It should be an excellent wheat and alfalfa soil.

When fertilizer treatment of soils becomes necessary because of either a low level of available nutrients to start with or because of exhaustive cropping, then recognition that plant nutrients in soils exist in several categories of availability, and that transformation from one category to another takes place at a rate dependent on a number of specific conditions, will be basic to any satisfactory program of determining and interpreting the nutrient status of the soils. Originally the nutrient status was determined for the most part by total analysis. Then when the results did not correlate at all with crop growth and fertilizer response, attempts were made to determine the more readily available portions of the nutrient elements. Because existing knowledge of the actual forms in which the nutrients exist in soils and of the manner in which they change from one form to another was meager indeed, progress was slow, and faith in the eventual success of soil testing was abandoned by many workers in this field over a long period of time.

Advances in knowledge during the past 25 years with respect to exchangeable bases, to types of minerals and compounds that exist in soils, and to conditions (including in particular the pH of the soil) which determine rate and type of transformation from one form to another have all contributed greatly to the development of better and better soil tests and more satisfactory interpretation of the results thus obtained. Today, soil testing is carried forth on a vast scale in many states and countries. In Wisconsin during the present year, several hundred thousand soil samples will be tested for pH, readily available phosphorus and potassium, and in some cases for available boron and other nutrient elements.

The determination of the pH of soils is now both simple and accurate. The result tells immediately whether or not it is advisable to add lime for the most satisfactory growth of various crops. It also tells when a soil is so highly alkaline that special treatment is needed to counteract the condition.

Recognition that three categories of availability of each plant nutrient should be considered and that transformation from one category to another takes place is of tremendous help in the interpretation of results of soil tests for potassium. For example, in the case of this element, it is now quite generally recognized that for the satisfactory production of general farm crops, a minimum level of 200 pounds per acre-plow-layer of readily available (category A) potassium should be present. However, if this drops, say to 100 pounds through cropping, it is a much more serious matter in a strongly podzolized soil of northern Wisconsin than in a mildly podzolized soil of similar texture in southern Wisconsin, because in the former there will be much less category B potassium present which will change to the A category with crop feeding. Also, if it is desired to raise the level of category A potassium to 200 pounds in both cases, it will take considerably more potash fertilizer in the strongly podzolized soil because of more rapid and greater transformation to the naturally depleted B category.

Although interpretation of results of soil tests for the other nutrient elements may vary considerably from that for potassium, nevertheless, as it now appears, the general pattern should be quite similar. To be sure, much more research is needed in connection with methods of determining the amounts and forms of the various nutrient elements present in soils in the three availability categories. With the knowledge and special analytical instruments now at hand and being developed, further progress in this field is certain to be rapid. Thus, soil fertilization and management to the end that soils be made still better mediums for crop growth will be carried forth with ever-increasing confidence and success.

SUMMARY

Soils may be considered and studied from two standpoints. First, they may be looked upon as being natural entities or bodies, just like rock formations or rivers, or even plants and animals, and then studied with respect to their origin, morphology, and classification without any specific regard to their agricultural use. This kind of consideration of soils constitutes the science of pedology, which provides a sound and scientific basis for soil classification and mapping. Although pedology is not concerned directly with soil fertilization and management, it does point the way to the basic system of soil classification needed in determining proper land use and crop adaptation.

In the second place, soils may be looked upon solely from the standpoint of being media for plant growth. As the title of this paper suggests, most of the discussion has been given from this standpoint.

It was pointed out that soils are not only three-phase systems in the ordinary sense of the physical chemist, but have in addition a "living phase" represented by bacteria and other microorganisms whose number in a thimbleful of fertile soil may exceed the number of people on this earth. The soil is, indeed, an intricate dynamic system, and to understand how a fertile soil may function as a well nigh perfect medium for plant growth requires much study and considerable imagination.

Not only must a good soil provide anchorage and aeration for plant roots, store and deliver enormous amounts of water to growing plants, but it must also serve as a frugal custodian of a dozen or more nutrient elements. If a soil were not frugal with its resource of nutrient elements, it would soon become so depleted of these elements that destruction by erosion would be certain and rapid because a protective vegetative cover would then fail to grow. The manner in which a soil functions as a frugal and judicious custodian of nutrient elements and how the amounts of these in various forms should be considered and may be determined is explained in considerable detail. The unfailing dependability of a soil serving as a medium for plant growth is beautifully summed up in verse by David Grayson as follows:

Why risk with men your hard-won gold?
  Buy grain and sow; your Brother Dust
Will pay you back a hundredfold—
  The earth commits no breach of trust.

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CHAPTER

3

The Activities of Cations Held by Soil Colloids and the Chemical Environment of Plant Roots

C. EDMUND MARSHALL

THE CONCEPT of the soil solution encounters difficulties as soon as ionizing colloidal constituents contribute appreciably. In the author's opinion, these difficulties become insurmountable if the soil solution is defined either according to Burd's recent dictum (2) in which the contributions of the colloidal constituents or ionizing surfaces are excluded, or according to earlier views identifying the removable soil solution (obtained by displacement or by pressure) with the soil solution in situ. On the other hand, a frank recognition of the complexities involved, together with the utilization of modern methods for determining cationic activities in colloidal systems, opens up the possibility of their resolution.

THEORY OF THE SOIL SOLUTION

In what follows, the soil solution will be defined as the complete external aqueous chemical environment of the plant root. This is a macro concept, that is, it involves the mean chemical environment for each ionic and molecular species present. In actual soils variations undoubtedly occur from place to place on a single root hair, but we are dealing here with averages taken over large numbers of points of contact and immense multitudes of ions. It is also a static concept, avoiding reference to changes with time except in so far as these may be determined experimentally by activity measurements. This is not so serious a weakness as might be supposed, since any future kinetic treatments of ionic transfer between soil and plant will inevitably need both a firm point of departure and a practical experimental method for observing macro changes with time. Both are now available.

Some simplifying assumptions will be made regarding the nature of the soil systems. Sparingly soluble salts such as calcium carbonate, tricalcium phosphate, and calcium sulfate will be omitted from consideration. The arguments used below can readily be modified to include them. The soil system will be assumed to consist of (*a*) an inert skeleton, (*b*) negatively charged soil colloids holding cations in a diffuse ionic atmosphere, (*c*) soluble salts, and (*d*) water.

Consider now a large mass of such an idealized soil at a given moisture content. Suppose that a small amount of solution is forced out by pressure, the removal causing no appreciable change in the original soil. Then the relationship between the concentration of soluble electrolytes in the solution thus expressed, and in the soil mass, is governed by the Donnan equilibrium. It can therefore readily be determined. This means that we can define the conditions under which the contribution of the ionizing colloids becomes important and can evaluate the relationship between the soil solution as defined in this paper, the "Burdian" soil solution, and the solution expressed from the soil.

To do this we must assume that single cationic activities have meaning and are measurable and must use certain simplifications in order to get an approximate over-all picture.

Consider potassium-saturated soil in the presence of potassium chloride solution and let a_c represent the activity of the potassium ions associated with the soil colloid, a_B the activity of potassium ions in the "Burdian" soil solution (i.e., due to potassium chloride alone), and a_E the activity of potassium ions in the expressed fluid which is free from soil colloids. According to the Donnan equilibrium we then have

$$(a_c + a_B)a_B = (a_E)^2 \tag{1}$$

assuming that chloride ions have the same activity coefficients as potassium ions. What we need is the relationship of a_E to a_B when a_c takes on different values in relation to a_B. It can easily be seen that for finite positive values of a_c, a_E is always greater than a_B. Let $a_E = qa_B$. Then by substitution it is easy to show that

$$a_c/a_B = q^2 - 1 \tag{2}$$

giving a well-defined value of q or a_E/a_B for each value of a_c/a_B. The curve is a parabola, and we can see the situation at a glance (Figure 1). Under the above assumptions the curve will apply to any electrolyte whose anion and cation have the same valency, and if several such are present simultaneously each can be dealt with. Thus, if we know a_c, the cationic activity of the soil colloid, we can calculate how large the salt content will be for any chosen ratio a_E/a_B. Alternatively, for any value of a_B, corresponding to the "Burdian" soil solution, we can determine how large the cation activity of the colloid needs to be for q to assume any value greater than 1.

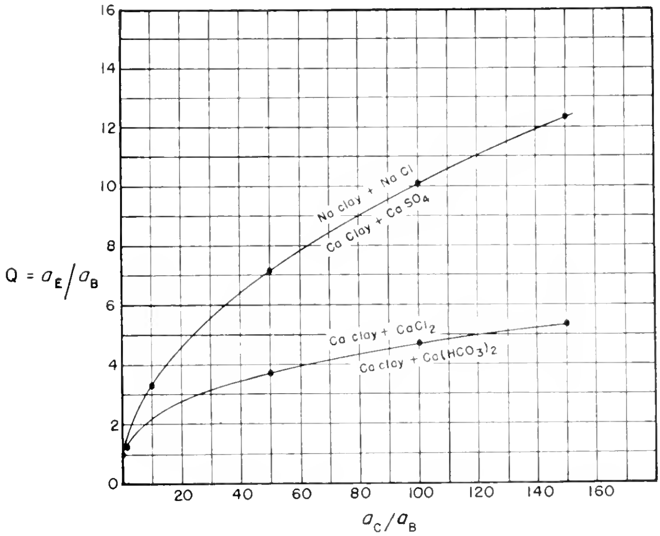

FIGURE 1. The Donnan equilibrium between soil colloids and expressed solution. For symbols see Table I.

Some interesting limiting cases arise. Obviously, if a_c is very much less than a_B, the "Burdian" soil solution becomes practically identical with the expressed solution and both are indistinguishable from the whole soil solution as here defined. Where appreciable colloid is present, it takes a high salt concentration to achieve this. Where the colloid content is very low, a much lower salt concentration will suffice. This explains why those who have worked with plant growth problems in highly saline soils and those who have had similar experience only in extremely light sands have been satisfied with a concept involving simple solutions of ordinary electrolytes. However, the great preponderance of productive agricultural soils are neither highly saline nor devoid of ionizing soil colloids.

Turning to the case in which a_c is much greater than a_B, we arrive at the formula for membrane hydrolysis, in which the compound under consideration is, in the above example, potassium hydroxide. Experimentally this is very simple, since $(a_c + a_B)$ is given by the cationic activity of the whole soil system, a_B by the hydroxyl ion activity of the whole soil system, and a_E corresponds to the potassium hydroxide in the expressed liquid. Thus, if we know the pH of the soil system and its cation activity, we can immediately determine the composition of the expressed liquid. The strength of the soil colloid as an electrolyte comes into this picture only through a_c, the cation activity of the colloid under the given moisture conditions. The expressed liquid will be alkaline, hence this equilibrium will be very sensitive to the presence of weak acids, such as carbonic, in the system. It is easy to see that such an acid will greatly increase the potassium activity in the expressed liquid. It does not do this, however, primarily by competition with the soil colloids, which are much stronger acids than carbonic, but indirectly through its effect on the Donnan membrane hydrolysis.

Similar considerations may be applied to other cationic constituents of soil colloids; as for instance, calcium-saturated soils in equilibrium with calcium chloride. For this case the equation corresponding to (2) is $a_c/a_B = q^3 - 1$, graphically represented also in Figure 1. Here again we reach the limiting condition for membrane hydrolysis when a_c is very large in comparison with a_B. The hydrolysis can be calculated from the equilibrium with respect to calcium hydroxide, using the appropriate Donnan equation. It will be a sensitive function of the pH of the soil system, since the hydroxyl ion concentration comes in as the second power.

Table I illustrates the interconnections of the three concepts of the soil solution. The three ranges chosen in each case for a_c cover the cases most likely to be encountered. Three chloride concentrations are included under a_B and in addition the situation in the absence of salt is examined, as it would be at the neutral point. Here the hydroxyl ion activity is 10^{-7} and $a_c + a_B$ is practically identical with a_c. a_E then represents hydroxide expressed from the soil. The effect of pH on the membrane hydrolysis is readily calculated from the Donnan equations. For potassium each increase in pH of one unit increases the potassium in the expressed liquid by $\sqrt{10}$. For calcium the corresponding factor is $\sqrt[3]{100}$.

TABLE I

The Operation of the Donnan Equilibrium in Soil Systems

Cation*	a_c	a_B	$a_c + a_B$	a_E	$q = a_E/a_B$
Potassium	10^{-2}	10^{-7}	0.0100	3.16×10^{-5}	—
		10^{-4}	0.0101	1.005×10^{-3}	10.05
		10^{-3}	0.0110	3.32×10^{-3}	2.32
		10^{-2}	0.0200	1.41×10^{-2}	1.41
	10^{-3}	10^{-7}	0.00100	1.00×10^{-5}	—
		10^{-5}	0.00101	1.005×10^{-4}	10.05
		10^{-4}	0.00110	3.32×10^{-4}	3.32
		10^{-3}	0.00200	1.41×10^{-3}	1.41
Calcium	10^{-3}	10^{-7}	0.00100	2.15×10^{-6}	—
		10^{-5}	0.00101	4.66×10^{-5}	4.66
		10^{-4}	0.00110	2.24×10^{-4}	2.24
		10^{-3}	0.00200	1.26×10^{-3}	1.26
	10^{-4}	10^{-7}	0.000100	1.00×10^{-6}	—
		10^{-6}	0.000101	4.66×10^{-6}	4.66
		10^{-5}	0.000110	2.24×10^{-5}	2.24
		10^{-4}	0.000200	1.26×10^{-4}	1.26

*a_c represents the cationic activity of the soil colloids.
a_B represents the cationic activity due to soluble salts alone in presence of the soil colloids.
a_E represents the cationic activity of the expressed solution.

It can clearly be seen from Table I that large differences exist between the expressed solution, the "Burdian" soil solution, and the whole soil solution as here defined. As was pointed out above, these differences will become even more marked in presence of carbonic acid.

CATIONIC ACTIVITIES IN HOMIONIC SOILS

To bring these aspects into sharper focus some concrete results of cationic activity determinations in various soil–water mixtures may be considered. Table II, taken from the data of Marshall and McLean (*16*), gives potassium and calcium activities on two homionic soils containing no added salts. The potassium and calcium ion activities were determined by clay membrane electrodes using techniques fully described elsewhere (*9, 10, 11, 14*). The fraction active is the ratio of the measured activity to the total concentration of the ion concerned. In true solutions it is known as the activity coefficient. The following conclusions then emerge from an examination of Tables I and II together.

TABLE II

Potassium and Calcium Activities Measured at Different Soil–Water Ratios on Homionic Soils

Soil	Soil: Water Ratio	Potassium		Calcium	
		Activity	Fraction active	Activity	Fraction active
Putnam silt loam (beidellitic)	3:4	18.3×10^{-3}	0.172	8.5×10^{-4}	0.016
	3:2	34.3×10^{-3}	0.161	16.4×10^{-4}	0.015
	3:1	58.0×10^{-3}	0.136	35.8×10^{-4}	0.017
Cecil clay (kaolinitic)	3:8	6.8×10^{-3}	0.267	15.4×10^{-4}	0.131
	3:4	12.1×10^{-3}	0.258	22.1×10^{-4}	0.094
	3:2	19.9×10^{-3}	0.211	32.8×10^{-4}	0.070

1. In spite of the fact that the clay colloids are weakly ionized, the cationic activities of salt-free soils in the moisture range where plants actually grow are significant. Even in the case of calcium we have activity values of the order of 3×10^{-3}, which is considerably greater than would be given by calcium carbonate in equilibrium with ten times the carbon dioxide pressure of ordinary air.
2. The actual activities obtained depend on the nature of the soil colloid present as well as on the amount.
3. The cationic activities are a sensitive function of the soil–water

ratio. This means that the chemical environment of the plant root changes greatly with changing moisture conditions in the soil.

4. Since different cations are ionized to different degrees, it becomes exceedingly important to determine all the factors involved. In a natural soil system with a mixture of exchangeable cations present, the relative activities may be quite different from the relative exchange quantities.

5. The expressed soil solution will be considerably different from the soil solution in situ at the low salt contents of nonsaline soils containing appreciable amounts of soil colloids. In many cases the cationic environment of plant roots will be provided chiefly by the soil colloids.

In view of these conclusions the detailed study of the electrochemical properties of soil colloids becomes of great importance to plant nutrition. The main results thus far obtained are reviewed below.

THE CATIONIC ACTIVITIES OF SOIL COLLOIDS

The colloids of soils may be divided into two main groups, inorganic and organic. In the inorganic group, the clay minerals are of predominant importance in contributing cations. The organic colloids are dominated by humified materials of an electronegative character having, in general, higher capacities for cation exchange than the clays. In a majority of fertile soils, the content of organic matter is less than that of the clay minerals.

The electrochemistry of these two groups of soil colloids had, until the advent of clay membrane electrodes, been developed through pH measurements and conductivity determinations. Potentiometric and conductometric titrations of the free colloidal acids, generally purified by electrodialysis, had led to the conclusion that both the clays and the humic materials were genuine colloidal acids, owing their acidic character to inherent structural features and not acquired by adsorption of soluble acids from solution. Many attempts were made to compare them with weak soluble acids by ascribing to them approximate dissociation constants.

Evidence on the exchange properties of these soil colloids also contributed to our knowledge. Exchange isotherms were obtained by measuring analytically the exchange equilibria for different proportions of colloid and electrolyte. These data, like those obtained potentiometrically, were generally interpreted on the assumption that all exchange cations of a given kind on a given colloid were held by the same bonding energy. Some evidence at variance with this simple concept gradually accumulated but was not regarded as sufficiently general to invalidate it.

Three main items may be mentioned. (*a*) Certain potentiometric titration curves of clays showed more than one inflection. (*b*) The last traces of exchangeable hydrogen were found, in general, to be extremely difficult to replace by metallic cations. (*c*) Hysteresis effects were found in certain exchange reactions; that is, the equilibrium was different when approached from different sides.

The logical consequences of a variation in the bonding energy of a given cation upon a given soil colloid were first adequately discussed by Jarusov (*4*), who presented clear experimental evidence that the replaceability of metallic cations varied with the degree of saturation. He emphasized also the importance of the nature of the accompanying ions in determining the replaceability of a given cation, thus foreshadowing the important work of Jenny and Ayers (5) on the complementary ion principle.

The work summarized below was made possible by the development in the Missouri Experiment Station, of membrane electrodes which, acting in a somewhat similar fashion to the glass electrode, render the determination of the activities of single cations feasible. The sequence of events was briefly as follows.

Thin plates cut from crystals of cation exchange minerals were first employed. Below a certain concentration they functioned as electrodes sensitive to a variety of cations. Their preparation was difficult. The next materials tried were clay films produced by the evaporation of colloidal suspensions of high base-exchange clays. By preliminary heat treatments these membranes were found to acquire good mechanical stability and at the same time their electrochemical properties were improved (*11*). In the case of the hydrogen montmorillonite, it was found that membranes could be prepared which were either sensitive both to mono- and divalent cations (pretreatment below 450° C.) or which were sensitive only to monovalent cations (pretreatment above 450°

C.). These membranes were used in the determination of the activities of K^+ (*12*), NH_4^+ (*13*), and Na^+ (*15*) when these cations were added as hydroxides to carefully purified acid clays. Thus, complete titration curves, involving both the hydrogen ion and the added cation, were obtained for the four clay minerals: montmorillonite, beidellite, illite, and kaolinite.

Divalent cations were then considered, calcium being the first investigated. Accurate procedures were established for the determination of calcium and magnesium activities (*10, 14*); these were later extended to barium (*3*). Results comparable to those given by the monovalent cations have now been obtained for montmorillonite, beidellite, illite, and kaolinite using magnesium, calcium, and barium (*3*).

In this way very interesting evidence on the ionization of the clays was afforded and, as mentioned previously, its extension from homionic clay suspensions to homionic soils offers no difficulties.

Attention was then directed to a more difficult problem, namely, the simultaneous determination of monovalent and divalent cations present together upon the clay. Calcium and potassium were investigated first. By means of high temperature selective membranes, the potassium activities in such mixtures were readily and accurately determined. The calcium estimation was subject to a considerably greater error but sufficiently reliable information was afforded to give an over-all picture. These potassium–calcium relationships have now been established for the following clay minerals: two montmorillonites, a beidellite, two illites, a kaolinite, a halloysite, and attapulgite (*1, 7*). In a similar way potassium–magnesium relationships are under investigation. Work is also under way on similar evidence for the different fractions of soil organic matter.

In reviewing the information already available, it has been apparent for some time that the complex nature of the complete titration curves of the clay minerals indicates that they hold single cations with a considerable range of bonding energies. It has proved possible to utilize the activity measurements to calculate these bonding energies. Thus, in addition to the pH curve and the cationic activity curve, we may derive also a bonding energy curve for each cation upon each clay. Where two cations are concerned we may express their mutual effects in terms of shifts in the bonding energy curves, comparing for instance, potassium-calcium with potassium-hydrogen systems and calcium-potassium with calcium-hydrogen systems.

Evidence obtained with single monovalent cations

The complexity of clay titration curves may be illustrated by the case of 2.8 per cent hydrogen bentonite and sodium hydroxide (Figure 2). Three curves are presented. A is the ordinary pH titration curve, characteristically smooth and with a single reasonably well-defined inflexion point. Curve B relates the sodium ion activities to the amount of base employed. Its complex characteristics are well established for

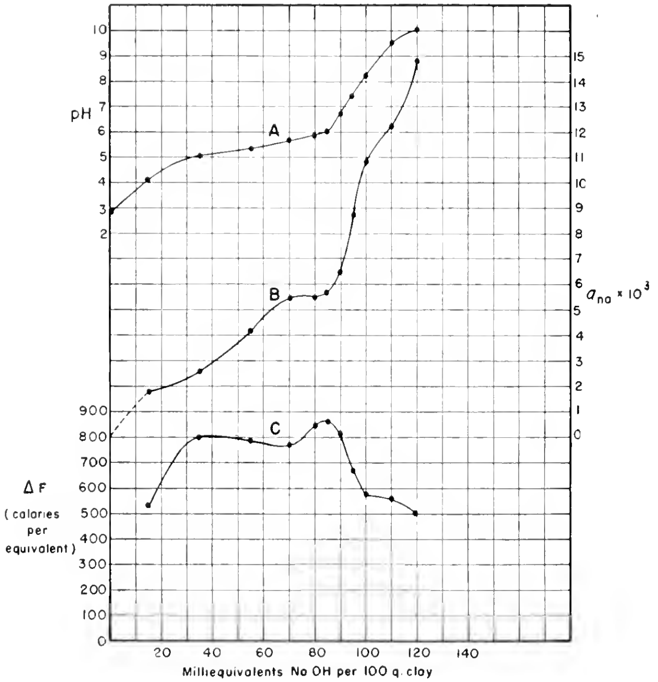

FIGURE 2. Clay titration curves for 2.8 per cent Wyoming bentonite with sodium hydroxide. *A:* pH titration curve. *B:* Sodium ion activity plotted against base added. *C:* Mean free energy of sodium ions plotted against base added.

this combination of clay and cation. C is a cationic free energy curve. At each point the free energy value is calculated from the actual activity and the total concentration of the cation concerned. The formula may be written

$$(\Delta F)_{\text{cation}} = RT \ln c_{\text{cation}}/a_{\text{cation}} \,.$$

The reciprocal of the cationic fraction active is therefore used in calculating $(\Delta F)_{Na}$, since a_{Na} (measured) $/c_{Na}$ (total) is the fraction active for sodium.

Corresponding to the sharp changes in slope in curve B, we find regions of rapidly changing free energy in curve C. The range, in mean free bonding energy of the sodium, runs from about 500 to 800 calories per mole ion. Similar curves are found for potassium and ammonium although the extremes are not quite so far apart. As regards the other three clays: beidellite shows many of the features of montmorillonite but with a considerably smaller fraction active corresponding to greater free energy values; illite gives somewhat simpler activity and free energy curves and high free energy values; kaolinite resembles illite in its curves, but the actual free energies are the least of any of the four clays. In making comparisons of one clay mineral with another, it is important to keep the total cation concentration approximately constant, since for each clay the fraction active and, hence, the cationic free energy varies considerably with clay concentration. A series of values of the fraction active is given in Table III for definite stages in the neutralization of the acid clays with bases. The table does not, of course, display all the variations in the fraction active shown by the complete curves. The following conclusions may be drawn.

1. If the state of each cation at the inflexion of the pH curve is taken as standard, then with decreasing degrees of saturation the fraction active falls, the rate of decrease being rapid down to 80 per cent saturation and slower between 80 per cent and 30 per cent. The bonding energy in the latter range runs from 20–50 per cent higher than that at the inflexion.

2. The fact that the fraction active is considerably less than unity suggests at first sight that we are dealing with weak electrolytes. However, the ionization is not appreciably changed by adding soluble salts with

a common cation (*12, 13*). The analogy with weak acids and bases therefore breaks down. These colloidal electrolytes do not readily adapt themselves to classifications based on the behavior of small molecules in true solution.

3. Kaolinite stands somewhat apart from the other three clays in its ionizing properties. The acid clay is much less dissociated than hydrogen montmorillonite, hydrogen beidellite, or hydrogen illite, yet the salts with monovalent cations are more dissociated. A structural reason can be suggested for this difference. Kaolinite has an electrostatically neutral framework, with ionization probably restricted to broken edges of the sheets. The other clays have negatively charged framework structures, the charges being distributed over the cleavage surfaces of the sheets. These charges are neutralized by mobile cations whose activities we measure.

TABLE III

Cationic Fractions Active for Four Clays at Definite Stages of Neutralization*

Clay	Percentage of Concentration	Equivalence, m.e./100	Cation	Fraction Active at the Following Stages of Neutralization		
				50%	75%	100%
Montmorillonite	2.8	100	sodium	0.377	0.258	0.381
	3.3	100	potassium	0.295	0.271	0.297
	3.0	100	ammonium	0.264	0.249	0.245
Beidellite (Putnam)	4.0	70	sodium	0.096	0.092	0.131
	4.6	70	potassium	0.122	0.105	0.149
	5.0	70	ammonium	0.053	0.067	0.098
Illite	10.0	28	sodium	0.073	0.076	0.123
	10.0	28	potassium	0.144	0.121	0.155
	10.0	28	ammonium	0.144	0.130	0.134
Kaolinite	10.0	3	sodium	0.248	0.264	0.336
	10.0	3	potassium	0.195	0.238	0.326
	10.0	3	ammonium	0.234	0.255	0.252

*Data from references *12, 13, 15*

Evidence obtained with single divalent cations

In comparing results obtained for divalent cations with those for monovalent, certain requirements as regards energy must be kept in mind. Suppose that two identical, neighboring, exchange spots release the same amount of energy when combining with two monovalent cations as they do with one divalent cation. The bonding energy per equivalent is therefore the same in the two cases. However, in terms of ions, the bonding energy per divalent cation is twice that per monovalent cation. From the logarithmic formula relating bonding energy per mole ion to the fraction active, one can readily see that the fraction active of the divalent cation would be the square of the fraction active of the monovalent cation. It is therefore to be expected that divalent cations will show very low ionization from silicate surfaces. Thus, if the fraction active for a certain monovalent cation is 0.1, then that for a divalent cation releasing the same energy per equivalent should be 0.01.

This is often found experimentally to be the order of magnitude of the relationship, allowing, of course, for variations due to cationic individuality as it may show itself in hydration or in geometrical relation to the silicate surface.

Figure 3 gives the curves, analogous to those of Figure 2, for hydrogen montmorillonite titrated with calcium hydroxide. The activity of calcium is seen to be almost constant over a considerable range, extending from about 30–70 per cent saturation with base. Then it rises very rapidly through the point of equivalence. This general behavior is found for all four clays (montmorillonite, beidellite, illite, and kaolinite) and for the three cations (magnesium, calcium, and barium) with some variation in the extent of the flat position and the subsequent rise. The actual values of the fraction active are summarized in Table IV.

Since calcium is the major exchange cation in most fertile soils, a great deal of practical significance emerges from a study of these figures. Acid soils, if less than 70 per cent saturated with calcium, may be expected to give a very low calcium activity. The lime required to bring the saturation up to 70 per cent will make relatively little difference as regards the chemical environment of the plant root. Between 70 and 100 per cent saturation the addition of lime greatly increases the calcium activity. This picture, of course, may need modification for soils in which organic matter plays a predominant role, but it accords very well with practical experience on soils high in clay. The comparison of kaolinitic soils with those dominated by members of the montmorillonite group is also extremely interesting.

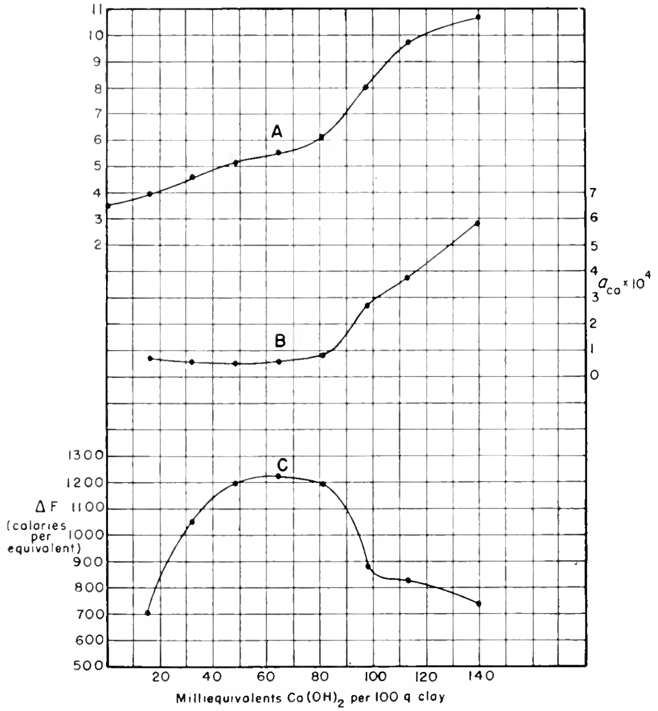

FIGURE 3. Clay titration curves for 1.07 per cent Wyoming bentonite with calcium hydroxide. *A:* pH titration curve. *B:* Calcium ion activity plotted against base added. *C:* Mean free energy of calcium ions (per equivalent) plotted against base added.

Under any given set of circumstances the actual value of the fraction active determines the mean bonding energy of the cations present and, hence, controls the ease with which they can be exchanged for other cations. It is entirely possible to have two soils, dominated by different clay minerals, which would give the same calcium activity but different bonding energies. For very small exchanges against hydrogen or other cations these would behave alike, but for moderate or large exchanges considerable differences would be apparent. Thus, in order to interpret the behavior of soils with regard to plant growth in the field, we should employ both the activities and the fraction active at various moisture contents. In addition we need control experiments designed to show how far the living root changes its external ionic environment as growth proceeds.

TABLE IV

Cationic Fractions Active or Four Clays at Definite Stages of Neutralization Using Divalent Cations

Clay	Percentage of Concentration	Equivalence, m.e./100 g.	Cation	Fraction Active at the Following Stages of Neutralization		
				50%	75%	100%
Montmorillonite	1.04	100	magnesium	0.0122	0.0086	0.0085
	1.07	100	calcium	0.0175	0.0172	0.0530
	1.07	100	barium	0.0036	0.0063	0.0235
Beidellite (Putnam)	4.8	70	magnesium	0.0059	0.0035	0.0068
	5.0	70	calcium	0.0040	0.0035	0.0081
	3.4	70	barium	0.0036	0.0045	0.0109
Illite	4.9	28	magnesium	0.049	0.030	0.023
	4.9	28	calcium	0.048	0.032	0.034
	4.9	28	barium	0.0036	0.0027	0.0087
Kaolinite	9.0	3	magnesium	0.0097	0.0057	0.062
	9.0	3	calcium	0.0134	0.035	0.092
	9.0	3	barium	0.0174	0.065	0.106

NOTE: The values for the equivalence have been arbitrarily chosen to correspond with those of Table III. Actually the inflexions on the pH curves are somewhat different for divalent cations than for monovalent. Data from reference *3*.

Evidence obtained with one monovalent and one divalent cation

Since we have perfected membranes of two types, those sensitive only to monovalent cations and those sensitive to all cations, it has proved possible, within limits, to determine one monovalent and one divalent cation in a mixture (7). This was regarded as particularly important because of the vast literature dealing with potassium–calcium relationship in plant nutrition. To clarify the potassium–calcium situation in soil colloids naturally seemed the first step.

If each of the cations concerned were held with a single bonding energy, then in a clay colloid saturated with a mixture of two cations we should anticipate that the fraction active for each should be constant. The activity for each cation would be directly proportional to the total amount present. On the other hand, with a range of bonding energies for each of the cations concerned, a variety of situations may arise, depending upon their quantitative relationship. Thus the fraction active of the one cation will become dependent upon the amount of the second ion present, as well as on its nature. In this sense one sometimes refers to the effect of one ion upon the activity or the fraction active of another. Actually, no direct effect of the nature of a steric hindrance, etc., need be present. The mutual relationships found are consequences of the energy differences of reactive spots on the silicate surfaces.

The importance of determining one cation in presence of another was realized as soon as membranes sensitive only to monovalent cations had been perfected. Measurements of potassium ion activities were made in a series of Putnam clay systems with differing proportions of calcium and potassium (*12*). It was concluded that in the middle range of base saturation (pH 5.5–5.7), there was little effect of calcium on potassium. We now know that this result is a special case and that it was largely due to the particular proportions employed. In general, as we shall see below, the potassium ion activity and the fraction active are greatly influenced by the proportion of calcium.

The total information on these relationships is now considerable, the following list of clay minerals having been investigated; two bentonites, Putnam clay (beidellite), two illites, halloysite, kaolinite, and attapulgite. One of the bentonites was reported on by McLean and Marshall (7), the other systems have been investigated here by S. A. Barber (Ph.D Thesis, 1949). The complete results, including studies of magnesium–potassium relationships by E. O. McLean, will be published elsewhere.

Three sets of measurements were carried out on each clay system. By titrating the acid clays with potassium hydroxide and with calcium hydroxide and determining potassium and calcium activities, two bases of comparison were established. Then mixed potassium–calcium systems were prepared (generally at a total saturation with bases of 100 per cent as judged by the inflexion on the pH curve), and the calcium and potassium ion activities were measured. Thus, complete information on the energy relationships of (*a*) hydrogen in presence of potassium or calcium, (*b*) potassium in presence of hydrogen or calcium,

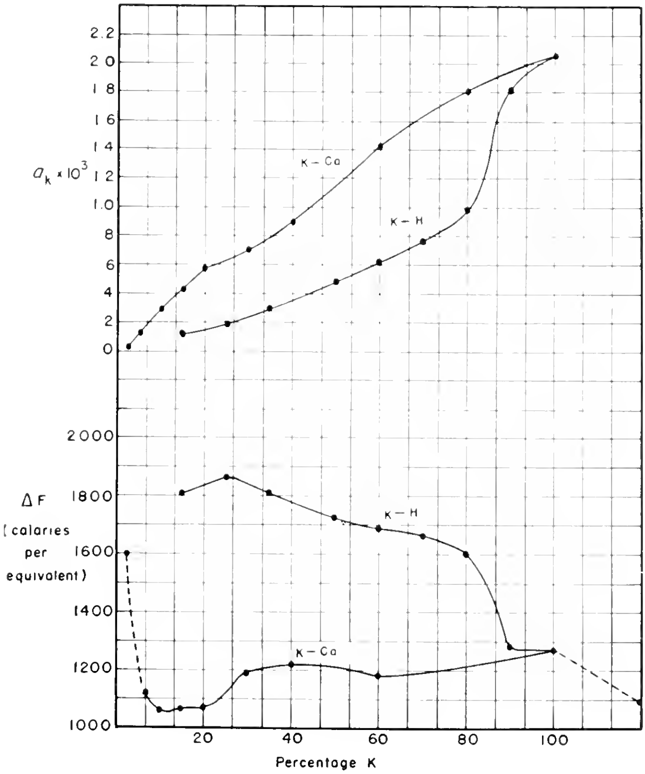

FIGURE 4. Comparison of potassium ion activities and mean free energy values in potassium–hydrogen and potassium–calcium clay systems (2.5 per cent Putnam clay).

and (*c*) calcium in presence of hydrogen or potassium can be worked out. The data obtained under (*c*) are limited by the difficulty of measuring very low activities of divalent ions in the presence of much higher activities of monovalent.

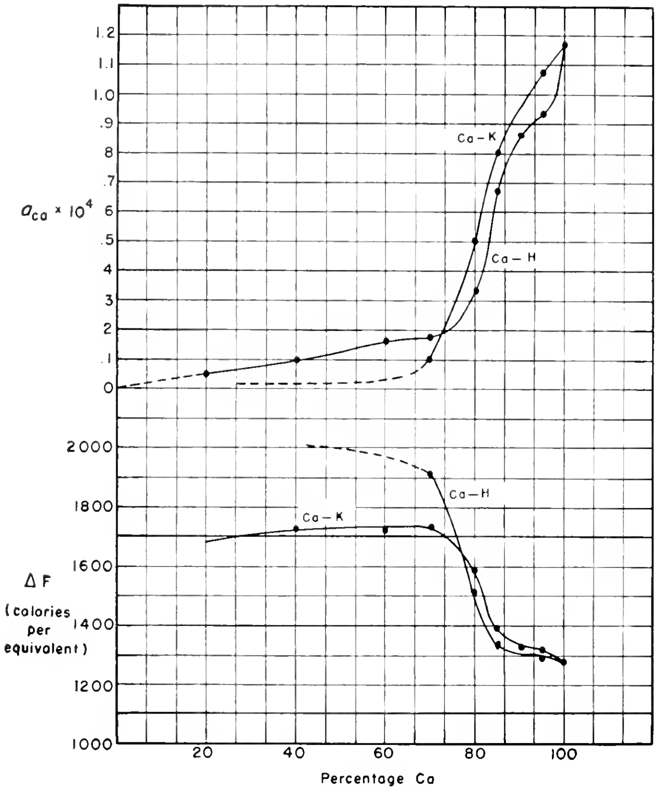

FIGURE 5. Comparison of calcium ion activities and mean free energy values (per equivalent) in calcium-hydrogen and calcium–potassium clay systems (2.5 per cent Putnam clay).

Figures 4 and 5 show how the mean bonding energy of potassium is affected by the presence of calcium replacing hydrogen and how that of calcium is affected by the presence of potassium replacing hydrogen. The particular clay used was Putnam (beidellite). It is evident that calcium greatly lowers the energy with which potassium is held. This result was obtained in varying degrees with all the clay minerals examined. The influence of potassium on the bonding energy of calcium depends greatly on the relative amounts of these cations and on the clay mineral under investigation.

The montmorillonite reported on by McLean and Marshall afforded a clear-cut case of the depression of calcium activity with increasing potassium and, hence, of an increase in the calcium ion mean bonding energy. The other clays are less regular and may show an increase over one part of the range and a decrease over another.

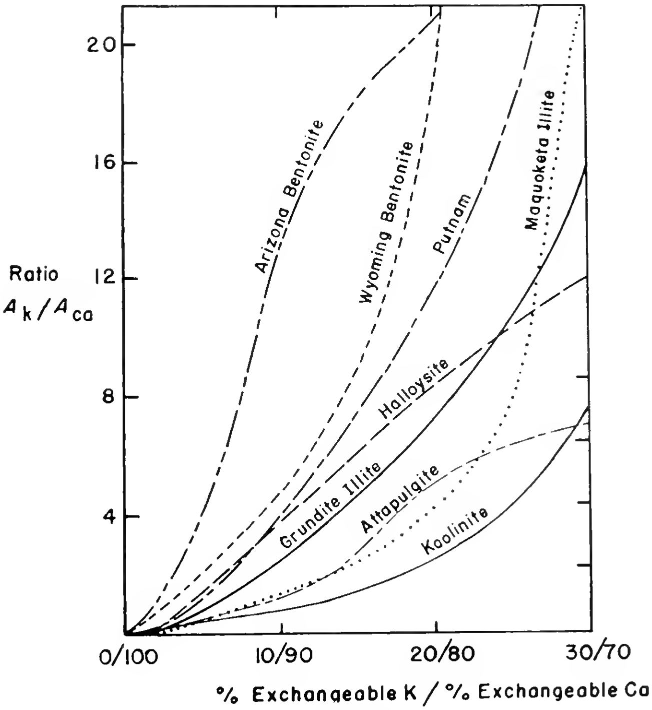

FIGURE 6. The potassium–calcium activity ratios for various proportions of the two exchangeable cations on a variety of clay minerals.

A general review of these potassium-calcium relationships may be obtained by plotting the ratio of the activities against the ratio of the total exchangeable quantities. This has been done in Figure 6 in which the activity ratio K/Ca is plotted vertically and the concentration ratio horizontally. It covers the range from 70–100 per cent calcium and 30–0 per cent potassium. The curves for the different clay minerals are spread over a wide range. The value of such a comparison can readily be seen by considering the case of a soil dominated by kaolinitic clay such as the Cecil in comparison with a beidellitic soil such as the Putnam. Let us suppose that both are 10 per cent saturated with potassium and 90 per cent with calcium. The ionic concentration ratio K/Ca is thus 0.2. Then in the kaolinite soil the activity ratio K/Ca is 0.8 while in the beidellitic soil it is 4.0. This means an enormous difference in the chemical environment of the plant root in the two cases.

Thus a considerable variety of factors play a part in establishing the mean chemical environment of the plant root. The nature of the colloids, their concentration, and the particular proportions of exchange ions present are all powerful in their effects. Much further investigation in this field is needed, but the experimental methods are well understood. They have proved reliable precisely in those ranges of activities in which plant roots live and grow.

CATIONIC ACTIVITIES AND PLANT GROWTH

From the composition of the nutrient solution used by plant physiologists it is possible to calculate approximate cationic activities. It has been shown that plants thrive over a considerable range of such activities, the range being widest where the cations concerned (usually K, Mg, and Ca) are in proper balance. So far as the author is aware, no studies have ever been published in which colloidal systems and true solutions have been compared at equal cationic activities as regàrds their effects upon plant growth. Such experiments are now entirely feasible. E. O. McLean (6) has made a preliminary study of this kind using montmorillonite clay with calcium and potassium together, and comparing this medium with dilute true solutions of the same activities. Soybeans were grown in both sets of systems at a prescribed series of calcium and potassium activities. In order to maintain constant conditions, frequent changes of the nutrient systems were made. The overall comparison of the effects of the solutions as compared with the colloidal suspensions was vitiated to some extent by the fact that the bentonite systems released some magnesium to the plants, whereas none was supplied in the true solutions. The plants grown on the calcium–potassium bentonite systems were uniformly larger than those grown on the true solutions but were of the same percentage composition as regards potassium and calcium. The most surprising feature of these experiments was that the systems giving $a_{Ca} = 0.7 \times 10^{-4}$ and $a_K = 1.8 \times 10^{-4}$ apparently provided ample supplies of both calcium and potassium, since larger amounts and varied ratios of calcium and potassium showed no increase in uptake. Future experiments will therefore have to be designed to give comparisons at even lower activities. The experimental methods for measurement will therefore be pushed to the utmost, since our membranes begin to deviate from the ideal behavior according to the Nernst equation below 10^{-4} molar in the case of potassium and below 10^{-5} molar in the case of calcium. We intend to repeat this type of experiment with better control of the colloidal medium since it is of such critical importance.

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CHAPTER

4

The Availability of Soil Anions

ROY OVERSTREET AND L. A. DEAN

THE AVAILABILITY of soil anions might well be considered as the state of being sufficient for the use of plants. Such a definition cannot readily be given a quantitative interpretation. Mineral nutrient availability usually embraces an integration of factors influencing the absorption of ions from soils and most studies concerned with availability give consideration to the factors which influence it. For example, organic matter and liming are believed to increase while fixation and lack of movement of phosphate ions tend to reduce phosphate availability. Indices of availability vary widely. Measurements such as the total amounts absorbed during the crop season and rates of absorption over a short period of time have been used.

Nitrates, sulfates, phosphates, borates, and molybdates are soil anions essential for plant growth. In addition, plant ash contains chlorides and silicates in important quantities. Available anions exist either in the soil solution or in a solid phase in equilibrium with this solution. This system contains—in addition to the anions absorbed by plants—carbonate, bicarbonate, hydroxyl, fluoride, humate, and sometimes arsenate and arsenite anions which frequently have an important bearing on the state of equilibrium of a soil water system.

By and large, consideration of anion availability has been restricted to a given plant nutrient. This discussion is an attempt to bring together the factors involved with the availability of soil anions in general.

ORIGIN AND DISTRIBUTION OF SOIL ANIONS

The essential elements absorbed by plants in the form of anions exist in soils in several discernible forms. These will be discussed under several different headings.

Primary minerals

Phosphorus, sulfur, boron, and molybdenum are components of many primary minerals. These elements may constitute a definite part of the crystal lattice, as in the case of apatite, pyrites, or tourmaline, or they may be occlusions or isomorphic substitutions. From the standpoint of an immediate source of anions for plant growth, these minerals are of little interest. Minerals such as apatite and tourmaline (*4*) when ground to pass a 100-mesh sieve do not supply phosphorus or boron at a rate sufficient for the normal growth of most plants when supplied in cultures in amounts comparable with which these elements usually are found in soils.

Organic compounds

Probably one of the most significant differences between the availability of soil anions and soil cations is that nitrates, sulfates, and phosphates are produced in soils as a result of biological activity. Also, the state of oxidation of nitrogen and sulfur is altered by biological activity.

It is generally assumed that most of the organic nitrogen in soils is protein in nature. The protein character of soil nitrogen has been variously studied. The carbon-nitrogen ratio is frequently used as an index of the nitrogen status of soils. This ratio (varies between 8 and 20) is a function of specific environmental conditions. The rate of nitrate formation in soils usually correlates more closely with the total nitrogen supply (*1*) than with measurements of the quality of the nitrogen compounds present in the humus.

A relatively large proportion of the total soil phosphorus frequently is associated with the soil organic matter. For example, Dean (*10*) measured the organic phosphorus content of 34 surface soils from widely separated parts of the world. The organic phosphorus content of these soils varied from 8 to 50 per cent of the total phosphorus and the carbon-organic phosphorus ratio from 44 to 160. The identity of the organic phosphorus compounds in soils is somewhat obscure. However, it is commonly believed that nucleic acids phytin and their derivatives (*7, 49*) account for a considerable part of the soil organic phosphorus. Incubation studies (*46*) have indicated that important amounts of the organic phosphorus are mineralized.

Evans and Rost (*16*) have shown the organic sulfur in many Minnesota soils to be an important part of the total soil sulfur. From 16 to 79 per cent of the total sulfur in these soils was found to be organic. The carbon–organic sulfur ratio for Minnesota soils varied from 87 to 451. There have been no studies on the specific nature of the organic sulfur compounds in soils. Little is known about the mineralization of soil sulfur in relation to the total supply of available sulfate.

Anions associated with the clay fraction of soils

Phosphates, boron, and molybdenum tend to accumulate in soils in association with the clay fraction. Analyses of clay separated from soils quite generally show a large part of the soil phosphorus and boron to be associated with this fraction. Analyses of soils have shown heavy ones to contain greater quantities of boron and molybdenum than those of light texture. What is known about the chemical nature of the anions associated with clays has been arrived at by indirect methods. Mineralogical and physical methods for the examination of clays have failed to show the presence of crystalline compounds of phosphorus or boron. Only through studies of the reactions between clays and solutions containing phosphates or borates—that is, phosphorus or boron fixation studies—is it possible to arrive at many of the properties of the phosphates and borates associated with the clay fraction of soils.

The availability of the anions associated with clays has not been satisfactorily understood. The equilibrium between the clay surfaces and the soil solution is considered by many to be a dominant factor controlling the availability of phosphates in soils. It is not improbable that the availability of borate and molybdate ions is similarly controlled.

Soil solution and soluble salts

Studies of the water-soluble material in soils date back to very early attempts to relate soil composition with nutrient uptake by plants. The inadequacy of the soil solution theory is probably the key to our present interest in nutrient availability. The amounts of soluble materials in soils range from very small amounts in the acid soils of the humid regions to the saline conditions of soils in the arid regions. The soluble materials in soils have been considered in detail by Reitemeier (*41*), Parker (*40*), Anderson (*2*), and others. It must be concluded that the soil solution for a given soil is highly dynamic.

The role of the soil solution in the absorption of anions by plants has never been wholly clarified. The amount of phosphorus, boron, and molybdenum present in the soil solution at any given time is inadequate for the nutrition of plants. In order that sufficient of these nutrients be available, it is necessary for one of two conditions to prevail; namely, that the soil solution be continuously renewed or that plants obtain these ions directly from the solid phase by contact feeding. Studies by Dean and Rubins (*11*) tend to minimize the importance of contact feeding as a means by which plants can obtain anions from soil surfaces. Studies by Volk (*50*) and Hunter and Kelley (*22*) on the absorption of ions from dry soils indicate that phosphates are not absorbed by plant roots from dry soils, whereas cations are absorbed. Studies on the effect of irrigation on the absorption of phosphates by sugar beets have shown absorption to be correlated with soil moisture. Judging from the rather scanty information available, it is not improbable that plants absorb anions from soils through the medium of the soil solution.

FACTORS INFLUENCING ANION AVAILABILITY

Soil organic matter and biological activity

Aside from being a possible source of available phosphorus, the organic matter itself is credited with having the capacity of increasing the availability of the soil phosphorus. Other reports show that humus reacts with rock phosphate, making it more available. Possible mechanisms by which humus makes inorganic soil phosphorus more available are not clear. Experimentally it has been shown by Steele (*43*) that less phosphorus is adsorbed by clays when humates are present in the system.

An interesting series of experiments was reported by Gerretsen (*17*) who showed that more phosphorus is absorbed from insoluble phosphates when in the presence of microbiological activity than from sterile cultures.

Soil texture

Soil texture can be shown to have various effects on availability. It is the common practice of European workers in this field (*15*) to take into consideration soil texture when evaluating soils for available nutrients. In general, light-textured soils need to contain higher amounts of readily soluble phosphorus than do heavy soils in order to have equal available phosphorus. At saturation, a unit volume of a heavy soil contains more soil solution than a light soil. The effect of this factor often has not been taken into consideration. Other things being equal, heavy soils fix more phosphorus. In so far as leaching or lack of leaching affects the amount of leaching and movement of ions in a soil, its texture influences availability.

Change in pH and lime status

A change in soil pH brings about changes in the equilibrium between the soil solution and the solid phase of soils. The following experiment by Dean and Rubins (*11*) illustrates this point well. Six samples of soil colloid were suspended in 200 ml. of water and continuously agitated. Varying amounts of monobasic sodium orthophosphate were added to the suspensions representing approximately 5, 10, 15, 20, 25, and 30 per cent of the saturation capacity. Collodion bags were introduced into each system, and at intervals small aliquots were removed for analysis. Twenty-eight days after the start of the experiment an equal amount of sodium hydroxide was added to each of the suspensions. In Table I are given the phosphorus concentration and pH values of the intermicellar liquids. These data show the magnitude of change of concentration with pH. Equilibrium constants for the sets of data are relatively close.

The effect of change in pH on the phosphorus, boron, and sulfate fixation will be discussed in a later section.

Liming of acid soils is reported to increase the availability of the soil phosphorus. Salter and Barnes (*42*) in summarizing the long time field experiments at Ohio concluded "These facts are interpreted as indicating an increase in the availability of native soil phosphorus as the reaction is made more alkaline up to about pH 7.5."

At one time it was believed that liming decreased the boron availability of soils. Subsequent work, however, indicates that if it does affect availability, it is because of the relation to boron fixation by soils (5). Other investigations have shown that boron fixed by soils under alkaline soil conditions is readily released by acidification.

TABLE I

Distribution of Phosphorus Between Clay Surfaces and the Liquid Phase of a Clay–Water System as a Function of pH, and Amounts of Phosphorus Added*

P Applied as Approx. Percentage Saturation	Composition of Intermicellar Liquid					
	22 days			35 days†		
	P	pH	K‡	P	pH	K‡
	p.p.m.			p.p.m.		
5	0.002	5.1	0.85×10^{-3}	0.059	6.9	1.6×10^{-3}
10	0.006	5.2	0.74×10^{-3}	0.069	6.7	1.9×10^{-3}
15	0.011	5.3	0.80×10^{-3}	0.079	6.6	2.1×10^{-3}
20	0.026	5.7	1.2×10^{-3}	0.345	6.6	0.61×10^{-3}
25	0.030	5.6	1.1×10^{-3}	0.262	6.8	1.8×10^{-3}
30	0.077	5.9	1.0×10^{-3}	0.533	6.5	0.51×10^{-3}

*Adapted from Dean and Rubins (*11*).

†Sodium hydroxide was added on the 28th day.

$$‡K = \frac{C_{OH}\ (\text{solution}) \times C_{H_2PO_4}\ (\text{surface})}{C_{OH}\ (\text{surface}) \times C_{H_2PO_4}\ (\text{solution})}$$

Robinson* has shown that crops grown on limed plots contained a greater concentration of molybdenum than the crops from adjacent unlimed plots.

Soluble salts

The concentration of phosphorus in the liquid phase of soils is dominantly influenced by the concentration and kind of salts present. The extent to which the concentration of phosphorus in the soil solution is increased by lowering the salt concentration can be demonstrated by comparing the phosphatic concentration of successive in-

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* W. O. Robinson, private communication.

crements of displaced soil solution. The effects hold for acid soils of the humid region as well as for calcareous and other soils of the arid regions. Mattson *et al.* (*32*) have found neutral salts to increase the uptake of phosphorus by plants.

Movement and leaching

The movement of anions in soils is dependent upon the species of ion involved, the extremes being a comparison of the highly mobile nitrate ions with the much less mobile phosphate ions. Losses of ions through leaching may be reasonably considered as a factor influencing availability. The upward movement of ions in soils should also be considered.

The very restricted movement of phosphate ions in soils may be a restriction on availability. This becomes increasingly apparent if the distribution of phosphorus is thought of as a mosaic throughout the soil mass. With a lack of migration of ions the effectiveness of a given root system would be reduced.

THE RETENTION OF ANIONS BY SOIL

From the foregoing discussion on the factors influencing the availability of soil anions, it may be seen that these factors are all in some way connected with the reactions that take place at the liquid–solid interface of soils. This section will deal with the kinds and mechanisms of these reactions.

Retention of phosphate ions by soil

The retention of phosphate by soils can be readily demonstrated by introducing soil into a solution containing phosphate ions and noting the decrease in concentration. This phenomenon is frequently termed "phosphate fixation." A review of this subject is available elsewhere (*13*). The mechanism of phosphorus retention has not been adequately established. Apparently no single mechanism is applicable to all soil conditions.

The older theories of phosphate retention by soils envision chemical precipitations. In acid soil systems iron and aluminum appear to be most likely agents, while in neutral or alkaline soils calcium and other divalent bases prevail. In acid soil systems solubility and plant growth studies point to possible inadequacy of a precipitation theory. In calcareous soil systems, however, a precipitation theory seems adequate.

Since the amount of phosphorus taken up by soils is proportional to the concentration, phosphorus retention has been ascribed to adsorption reactions. Adsorption is the tendency to concentrate at the interface. The term is general and may include several kinds of surface reactions. It does not carry any implication relative to the binding forces. Observations have shown that some of the phosphorus retained by soils is more tightly held than others. For example Mattson and Karlsson (*31*) have distinguished between *colloid-bound* phosphorus, a nondiffusable structural unit, and *saloid-bound* phosphorus, a diffusable ionic atmosphere held as compensation to ions of opposite charge.

The substitution of one ion for another or a metathetical reaction involving chemical forces and affinities is a means by which anions in solution may become associated with the solid phase or adsorbed. Thus, phosphate retention may be considered as the exchange of H_2PO_4 ions in solution for OH ions associated with the solid phase. An increase in pH of the system is associated with this reaction; conversely, phosphates of soils may be displaced by hydroxyl, fluoride, or arsenate ions (*12, 14, 45*).

Isotopic exchange studies (*30*) between $P^{32}O_4$ ions introduced into the soil solution and $P^{31}O_4$ ions associated with the solid phase have indicated that only a small proportion of the phosphate ions retained by soils are readily exchangeable with similar ions in the liquid phase, whereas similar studies (*6*) have shown that the exchangeable calcium ions were all in equilibrium with the calcium ions in the liquid phase.

The phosphorus retention by soils, for the most part, is restricted to the clay fraction. However, a large number of minerals common to soil exhibit, when finely ground, a capacity to fix phosphate.

In acid soils particular attention has been given to the hydrous oxides of iron and aluminum and to the clay minerals. Maximum retention by these materials usually occurs in the pH range of 4 to 6. Soils treated to remove the iron oxide invariably show a reduction in their capacity to fix phosphorus (*47*).

Retention of chloride and sulfate ions by soil

The retention of chloride and sulfate ions by soil is not as readily apparent as the phosphate retention. The mechanism by which these ions are retained in acid soil systems in some respects parallels that for phosphate.

Toth (*48*) measured the adsorption of chloride by clays before and after the free iron oxides had been removed (see Table II), and found the untreated clays to adsorb this ion. The decrease in chloride adsorption with increasing pH indicates the possibility that under acid conditions Cl ions replace OH ions associated with the free iron oxides.

TABLE II

Adsorption of Chloride Ion by Untreated and by Deferrated Colloids*

Colloid	Cl^- added as $HCl + NH_4OH$	Free Iron Oxide Content		pH of Supernatant Liquid		Cl^- Adsorbed	
		Original	Deferrated A	Original	Deferrated A	Original	Deferrated A
	m.e./10 g.	per cent				m.e./10 g.	
Cecil	20.00	—	—	3.70	3.70	0.320	0.000
	20.00	12.32	1.44	2.00	2.10	0.480	0.000
	20.00	—	—	1.80	1.80	0.520	0.020

*Adapted from Toth (*48*)

Barbier and Chabannes (*3*) studied the retention of sulfate ions in soils and reached the following conclusions.

1. Sulfate ions are retained by soils more strongly than Cl ions but less strongly than PO_4 ions.
2. Calcium ion favored the retention of SO_4 ion independently of the precipitation of $CaSO_4$.
3. Soils of average composition contain 10–20 mg. of sulfur per kilogram in the adsorbed condition.

Reitemeier (*41*), Kelley (*28*), and others have suggested sulfate adsorption to explain the increases in soluble sulfate on dilution of some soils.

Retention of boron by soils

Apparently the mechanism for retention of boron by soils does not parallel that of the other soil anions (Cl, SO_4, PO_4). Boron retention is lowest in acid soils, but increases rapidly in the range pH 6–10. Olson and Berger (*35*) have shown that fixation is not affected by calcium addition except when this addition influences the soil pH. Boron fixation by soils is readily reversible. Clays which have had the free iron and aluminum oxides removed from them fix more boron than untreated samples.

Negative anion adsorption

There is accumulating evidence that in neutral and alkaline soils there is negative adsorption of nitrate and chloride ions. Reitemeier (*41*) and others have observed decreases in soluble nitrate and chloride in soils on dilution. In other words, there are lower concentrations of these ions in the solution immediately surrounding soil particles than at some distance from them. It has been suggested that this phenomenon may be caused by the insolubility of nitrate and chloride in the "unfree" or bound water. Another concept of the mechanism of this negative adsorption is that it is caused by a diffused anion swarm, the distribution of which depends on the Donnan distribution principle.

ABSORPTION RATES AS A MEASURE OF ION AVAILABILITY

A widespread experimental method for the study of ion availability is the measurement of absorption rates with selected test plants, or plant tissues under varying conditions in the soil or culture medium. While providing a facile means for investigation, this approach, in order to be productive, requires an adequate comprehension of the absorption process in plants and a knowledge of the physical chemistry of ions in soil. Unfortunately, this essential knowledge is not available to us, although a number of pertinent facts begin to appear. It will be our purpose in the following sections to list the established facts concerning ion absorption in plants as well as certain physical-chemical observations regarding ions in soil. Particular attention will be paid to anions, although the majority of the considerations apply to cations as well.

Salient features of the ion absorption process in plants

No mechanism proposed thus far for the accumulation of ions by plant cells has received universal acceptance. Moreover, it will not be our purpose here to describe in detail the various mechanisms that have been put forth nor to judge them. On the other hand, we wish to describe a body of uncontested observations concerning the process which has been contributed to by a number of groups during the past 20 years. These groups include those associated with S. C. Brooks, R. Collender, D. R. Hoagland, H. Lundegårdh, W. J. V. Osterhout, R. N. Robertson, and F. C. Steward. This information can be outlined as follows.

1. The ion absorption process requires the expenditure of energy by the plant (isotopic exchanges excepted). No ion accumulation occurs in the absence of respiration and other metabolic activities such as protein synthesis, etc. In general, the process is an attribute of tissues capable of growth. When the metabolic activity of the plant is inhibited by reduced oxygen tension, lowered temperature, or poisons, ion accumulation is likewise inhibited. This is true for the accumulation of both anions and cations.
2. The ion absorption process is an exchange process. Predominantly cations are absorbed in exchange for H-ions of the plant and are released to the culture medium. Anions are absorbed in exchange for OH^- or HCO_3^- which are released to the culture medium. The evidence indicates that no ion passes in or out of a healthy plant except by exchange for another ion.
3. Ion accumulation is to a large extent selective. Due to the exchange character of the process, anions can enter the plant independently of cations and vice versa. Also, ions are not absorbed at the same rates. In general, the cations K^+, NH_4^+, Rb^+, and Cs^+ are rapidly accumulated while Ca^{++}, Mg^{++}, and Ba^{++} are much more slowly taken up. The anions NO_3^-, Br^-, and Cl^- are usually rapidly absorbed. The anions SO_4^{--} and $H_2PO_4^-$ are moving rather slowly; the anion HCO_3^- is apparently not absorbed at all.
4. With electrolyte solutions, and below concentrations of the order of 0.005 *N*, the rate of accumulation of an ion is dependent on its concentration in the culture medium.

The above-listed facts were essentially all that were firmly established concerning the ion absorption process before the advent of artificially prepared radioactive elements. The absorption process was known to be an exchange reaction which could be written formally as follows.

For cations,

$$R \cdot H + K^{+} \longrightarrow R \cdot K + H^{+}$$

and for anions,

$$R' \cdot OH + Cl^{-} \longrightarrow R' \cdot Cl + OH^{-}$$

where R and R′ symbolize the plant root. It was not known whether the combinations R·K and R′·Cl represented a chemical binding or some physical entrapment. With the introduction of the use of radioactive isotopes, certain important advances have resulted in our comprehension of the fixation of ions by plants. These advances have largely resulted from the study of isotopic exchange reactions.

The significance of isotopic exchange reactions in the study of the absorption process

The study of isotopic exchange reactions has been employed extensively by chemists to determine the nature of chemical binding. The most general expression for an exchange reaction may be expressed by the following equation, where A^* represents the tagged or radioactive isotope and A represents the untagged or stable isotope:

$$A^*Y + AX \rightleftharpoons AY + A^*X$$

Starting with A^*Y, measurements of the rate of exchange can be accomplished by noting the appearance of A^*X on the right-hand side of the equation. This rate is indicative of the type of chemical combination between A^* and Y. In general, if A^* and Y are held together by electron pair bonds, the exchange reaction does not proceed unless some side reaction is possible that involves a breakage or elimination of the covalent bond. If the bond between A^* and Y is electrovalent in character, the reaction ordinarily proceeds very rapidly, provided the ions entering into the reaction are physically accessible for exchange.

Isotopic exchange reactions were used for the first time to study the nature of the combination of inorganic elements with plant roots by Hevesy in 1923 (*18*). Hevesy studied the rate of the reaction

$$R \cdot Pb^* + Pb^{++} \longrightarrow R \cdot Pb + Pb^{*++}$$

where R symbolizes the plant root. Hevesy found that with *Vicia faba* plants, complete isotopic equilibrium was attained in 24 hours between absorbed lead and lead in the culture solution. From this fact he concluded that lead is held in the plant in the form of a dissociable saline compound, that is, by electrovalent forces.

At a much later date (*19, 20, 21*) Hevesy and his co-workers carried out similar experiments with radioactive phosphate. They concluded that in the case of a number of plant parts the phosphorus was held by electrovalent forces, presumably as inorganic phosphate, although in the case of a yeast sample it was probably held by electron pair bonds —possibly as hexosephosphate or adenylphosphoric acid.

In 1938 Jenny and Overstreet (*24*) studied the rate of the reaction

$$R \cdot K^* + K^+ \longrightarrow R \cdot K + K^{*+}$$

using potassium clays and potassium chloride solutions for the outside media. They found that although the reaction proceeded at a measurable rate, only about 10 per cent of the K^* initially held by the roots underwent exchange in a period of 24 hours.

These studies were soon followed by similar experiments with radioactive sodium, rubidium, potassium, bromine, and phosphate by Jenny, Overstreet, and Ayres (*26*), Mullens and Brooks (*33*), Overstreet and Broyer (*38*), and Broyer and Overstreet (*9*). In brief, the experiments showed that all the above-mentioned elements are held by the plant in an exchangeable form, and therefore most likely not entirely by electron pair bonds. On the other hand, a great diversity was noted in the rates by which individual elements underwent exchange. This indicated considerable variation in the strength of binding of the different elements in the plant.

Recently, the exchange of ions at extremely low concentrations between barley roots and culture solutions has been studied by Overstreet and Jacobson (*39*) and Jacobson and Overstreet (*23*). The exchange behavior of Rb^+, $H_2PO_4^-$, Sr^{++}, and I^- at concentrations of the order of 10^{-9} molal was studied. The advantages of such solutions are (*a*) freedom from osmotic effects and (*b*) extreme degree of sensitivity, enabling experiments to be carried out with single root tips for as short a time as 30 seconds. The isotopic exchange curves on individual apical segments were determined at temperatures near 0° C. in order to avoid complications due to metabolic activity.

The experiments showed that the isotopic exchange curves for the various ions were characteristically different. This fact will be apparent from Figure 1, which gives the curves for Rb^+, Sr^{++}, $H_2PO_4^-$, and I^-.

Also, it was found that in each case the isotopic exchange curve was characteristic of the living root. With ether-killed tissue, all the elements were released very rapidly by isotopic exchange. This point is illustrated in Figure 2 for the case of I^-.

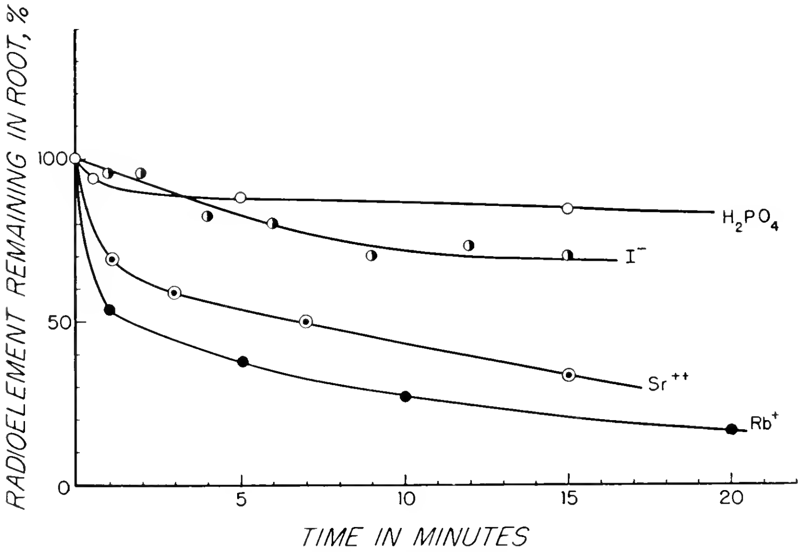


FIGURE 1. Graphs showing the release at 0° C. of P^{32}, I^{131}, Sr^{85}, and Rb^{86} by apical root segments in exchange for the inactive isotopes in the culture solution. Apical segments of roots (2 cm.) containing the absorbed radioelements were placed in 0.005 N solutions of $H_2PO_4^-$, I^-, Sr^{++}, and Rb^+, and counted at selected intervals to determine the loss of the radioactive elements by exchange. The percentage of the initially absorbed activity remaining in the root is plotted against time.—Overstreet and Jacobson (38), and Jacobson and Overstreet (23).

A third point of interest was the fact that, at least in the neighborhood of 0° C., the different elements showed rather widely different longitudinal absorption patterns in the root tips. That is, for example, regions in the tissue which absorb $H_2PO_4^-$ very rapidly do not necessarily absorb I^- rapidly. This finding is illustrated in Figure 3.

In general, the isotopic exchange experiments seem to indicate that the combinations R·K, R·Na, R′·Cl, R′·I, R′·H_2PO_4, etc., represent chemical attachments. It would be extremely difficult to interpret the two-way movement of ions characteristic of the isotopic exchange reactions on the basis of a mere physical entrapment.

It must be concluded further in the light of the exchange experiments that the various combinations represent either different chemical entities or at least similar complexes with widely varying energies of formation.

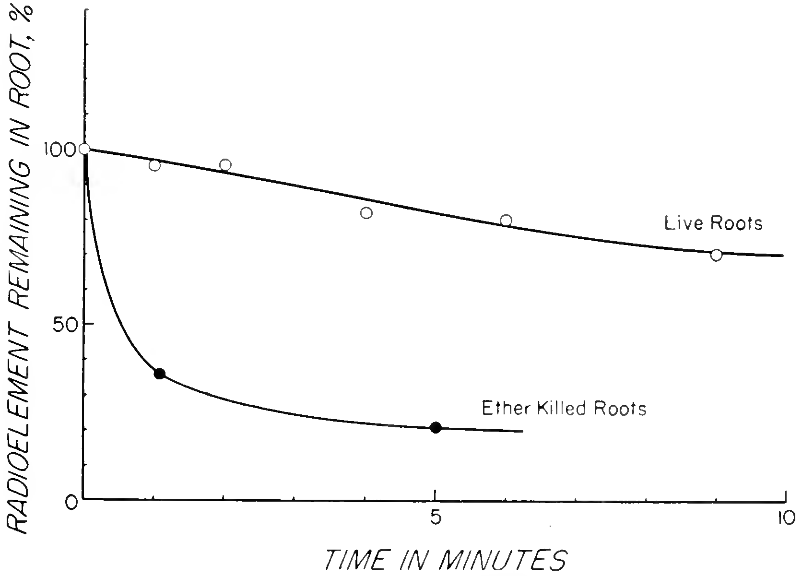


FIGURE 2. Graphs showing the exchange for inactive isotopes of I^{131} at 0° C. for live and ether-killed roots. The percentage of the initially absorbed activity remaining in the root is plotted against time.—Jacobson and Overstreet (23).

Finally, it must be concluded that the binding complexes are rather labile, since the mere injury or killing of the tissues is sufficient to destroy them.

The idea that ion absorption is chemical in nature is not in conflict with a number of absorption theories that have been put forth—some of long standing. Some of these postulations are of particular interest here.

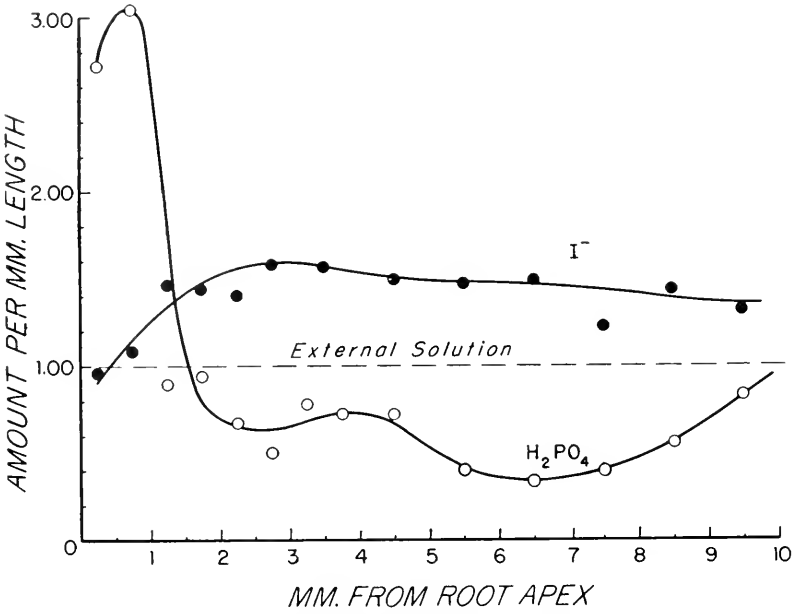


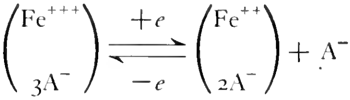
FIGURE 3. Graphs showing the distribution of absorbed P^{32} and I^{131} in barley roots as a function of distance from the root apex. The dotted line labeled "external solution" corresponds to the activity of a volume of the bathing solution equal to that of 1 mm. of root segment and is arbitrarily given the value of 1.00 in the graph. All other amounts are given in terms of the value of the external solution.—Overstreet and Jacobson (*38*), and Jacobson and Overstreet (*23*).

Hypotheses concerning the chemical nature of R·H and R′·OH

On the basis of certain investigations dealing with the mechanism of ion accumulation by plant cells, Osterhout (*36*) postulated that the plant substance R·H may be similar in its properties to some of the aromatic alcohols. Osterhout was successful in constructing an artificial model of a cell which consisted of two electrolyte solutions separated by a layer of potassium guaiacolate. By maintaining a difference in hydrogen ion concentration between the electrolyte solutions, he was able to show an accumulation of K-ions in the solution highest in H-ions. No hypotheses were made concerning the substance R′·OH.

On the other hand, Brooks (*8*) became interested in the role of amino acids in plant cells. He was led to the theory that the properties of both of the substances R·H and R′·OH were inherent in the amino acid molecule; the H^+ of the –COOH being exchangeable for cations and the OH^- of the $-NH_3OH$ groups being exchangeable for anions. By postulating an orientation of amino acid molecules in the protoplasm or plasma membrane (mosaic arrangement) Brooks was able to envisage the entry into the cell of both cations and anions along different paths by a series of ion exchanges involving H^+ and OH^-. At a much later date Steward and Street (*44*), on the theory that ion accumulation is intimately tied up with protein synthesis in the plant, speculated that R·H and R′·OH may correspond to the acidic and basic groups of certain phosphorylated energy-rich nitrogen compounds in the protoplasm.

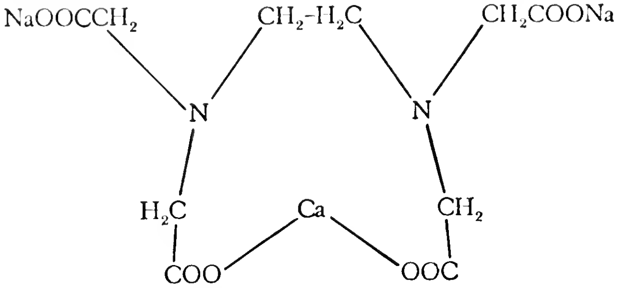
Very interesting speculations regarding the nature of R·H and R′·OH are embodied in Lundegårdh's theory of "anion respiration" (*29*). According to Lundegårdh there is little difficulty in accounting for the properties of R·H in plants, since the protoplasm as a whole is negatively charged and contains appreciable quantities of substances with comparatively strong acid properties. Cations in the culture medium therefore exchange for H^+ ions in the plasma membrane and proceed inward through the protoplasm by exchange along paths or tracks of substances of acid dissociation. No special energy would be required for this type of transport. For these reasons Lundegårdh's theory is chiefly concerned with the nature of R′·OH, that is, with the means by which anions are absorbed and moved within the plant. Accordingly, Lundegårdh conjectures that ". . . the Fe-ion in the hemin group of a respiratory enzyme is indeed well suited to effect an anion transport, according to the following scheme



$$\begin{pmatrix} Fe^{+++} \\ 3A^- \end{pmatrix} \underset{-e}{\overset{+e}{\rightleftharpoons}} \begin{pmatrix} Fe^{++} \\ 2A^- \end{pmatrix} + A^-$$

"The trivalent Fe-atom attracts one more anion than the bivalent Fe-atom. If the enzyme system constitutes a structural unit, in which electrons move from one atom to another in the next molecule . . . the conditions are given for a transport of anions. The change of valency proceeds as an electron wave, if the enzyme system lies parallel to a redox gradient. If the molecules of the enzyme are arranged in such a way that they serve as a boundary between two media of different redox potentials, owing to the wavelike proceeding oscillation of the Fe-valency, they will transport anions from the medium with higher oxydation power (Fe^{+++}; ox-side) to the medium with lower oxydation power (Fe^{++}; red-side). The anions are transported in opposite direction to the electrons. . . . The transference of an electron between two Fe-atoms moves one anion from the oxidized to the reduced stage."

On the basis of the character of the isotopic exchange curves, Jacobson and Overstreet (23) were led to conclude that the plant complexes $R \cdot K$, $R \cdot Rb$, $R \cdot Sr$, $R' \cdot H_2PO_4$, $R' \cdot I$, etc., probably do not represent combinations of the ordinary electrovalent type. They suggest that cations may be bound in plants in the form of chelated complexes. In this class of structure the metallic ion is attached at two or more points in the same molecule and one of the bonds is frequently coordinate in nature (see Yoe and Sarver, *Organic Analytical Reagents,* John Wiley and Sons, 1941). Many plant substances such as proteins, amino acids, and organic acids are known to form chelated compounds, particularly with polyvalent cations. Chelated structures in general are rather stable arrangements and metallic elements so bound do not readily undergo isotopic exchange. An example of this class of compound is given in the proposed structure for the calcium complex of ethylene-diamine-disodium-tetraacetate:



The foregoing outline of hypotheses concerning the nature of the plant substances $R \cdot H$ and $R' \cdot OH$ is by no means exhaustive. Nevertheless, it is hoped that it gives a picture of the present state of uncertainty concerning these compounds and indicates a wide field for future researches.

Information concerning the reversibility of absorption reactions

As will be emphasized in a later section, a knowledge of the reversibility of ion absorption processes is essential for the formulation of soil factors affecting ion availability. Here again our information is very meager. In a study of the effects of suspensions of hydrogen clays on barley roots, Jenny and Overstreet (*24*) obtained results that perhaps serve as evidence for the reversibility of the reactions

$$R \cdot H + K^+ \longrightarrow R \cdot K + H^+$$

and

$$2\,R \cdot H + Ca^{++} \longrightarrow R_2 \cdot Ca + 2H^+$$

The plant roots were observed to lose significant fractions of their potassium and calcium contents after a few hours of contact with dilute hydrogen-bentonite suspensions. Evidently this loss was not the result of permanent injury, since subsequently it could be shown that the tissue absorbed normally from standard nutrient solutions.

In so far as can be determined, evidence for the reversibility of anion absorption is even less substantial than in the case of cation absorption. In some unpublished work by J. M. Heslep, plant roots were placed in suspensions of clays of the kaolinite type. In a few hours significant amounts of phosphate were observed to leave the roots and become fixed on the clay. This may be taken as presumptive evidence for the reversibility of the reaction

$$R' \cdot OH + H_2PO_4^- \longrightarrow R' \cdot H_2PO_4 + OH^-.$$

A series of experiments testing the reversibility of absorption reactions in general are being undertaken in the Divisions of Soils and Plant Nutrition at Berkeley.

The effects of concentrations and activities on absorption rates in nutrient solutions

Ion-absorption experiments conducted with flowing nutrient solutions, or with nutrient solutions maintained at constant composition, definitely show that up to concentrations in the neighborhood of 0.001 N, the absorption rate of an ion is a linear function of its concentration in the culture medium (*24, 37*). Since, in nutrient solutions as ordinarily used, the activities of ions are not appreciably different from their molalities, it is not possible to discern in these cases whether the rates of absorption of the ions by plants are determined by the concentrations or by the activities of the ions. This point, while perhaps not of great importance in the study of plants growing in nutrient solutions, becomes a question of major concern in the estimation of ion availability from soil systems. This becomes evident when the physical chemistry of soil ions is considered.

Some conclusions regarding the activities of soil ions

The characteristic chemistry of soil ions becomes apparent when a soil suspension or gel in equilibrium with its filtrate or "soil solution" is considered. This approach has practical as well as theoretical implications, since many soil fertility tests are based on the chemical composition of the soil solution rather than of the whole soil. Such an equilibrium system can be represented as outlined in Figure 4.

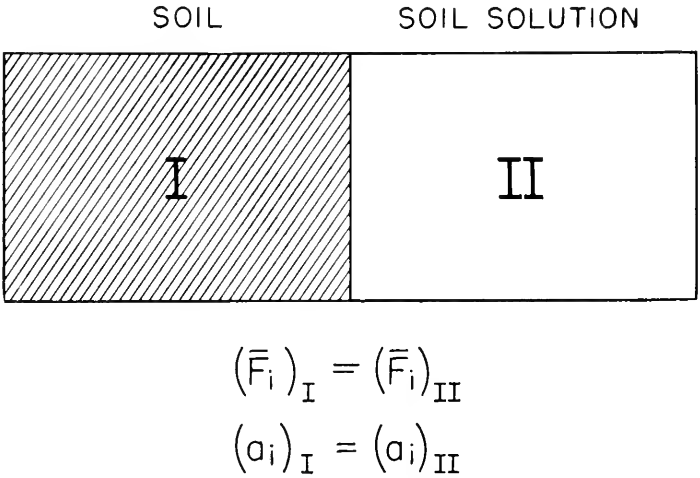


FIGURE 4. Diagram showing a soil suspension or gel in equilibrium with its filtrate or soil solution.

Phase I contains soil particles and ions such as Ca^{++}, Mg^{++}, Na^{+}, and K^{+} in the adsorbed state. Also it will contain the aforementioned cations and anions such as Cl^{-}, HCO_3^{-}, NO_3^{-}, $H_2PO_4^{-}$, and SO_4^{--} in the free or unadsorbed state. Phase II is purely an electrolyte solution which contains no soil particles.

We may now consider the conditions for equilibrium between Phase I and Phase II. According to a law of thermodynamics, the partial molal free energy ($\overline{F}$) of any component is the same in all phases of a system of phases at equilibrium. That is, for example,

$$(\overline{F}_{KCl})_{I} = (\overline{F}_{KCl})_{II} \text{ at equilibrium.} \qquad (1)$$

This thermodynamic law is perfectly general in character and applies for the case of charged ions as well as for the case of uncharged components. For this reason we can also write

$$\begin{aligned}
(\overline{F}_{K^+})_{I} &= (\overline{F}_{K^+})_{II} \\
(\overline{F}_{Cl^-})_{I} &= (\overline{F}_{Cl^-})_{II} \\
(\overline{F}_{H_2PO_4^-})_{I} &= (\overline{F}_{H_2PO_4^-})_{II} \\
&\cdots\cdots\cdots
\end{aligned}$$

Since we know that the concentrations of individual ions in our system may be quite different in the two phases, it should be borne in mind that the partial molal free energy of an ion in such a system may be a function of factors other than concentration such as electric potential or interaction with surfaces.*

In conformity with the original definition of activities, we can define the activity (a) of an individual ion in our system by means of the equation:

$$\overline{F}_i - \overline{F}_i^0 = RT \ln a_i$$

where $\overline{F}_i^0$ represents the partial molal free energy of the ion i in an arbitrarily chosen standard state. Consequently, we may write

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*It should be noted that in this approach the partial molal free energy of an individual ion is identified with the "escaping tendency" of the ion. It is identical with the *electrochemical potential*, $\overline{\mu}$, of another treatment and nomenclature (for example, see *The Physical Chemistry of Electrolytic Solutions* by H. S. Harned and B. B. Owen, New York, Reinhold Publishing Company, 1943, page 315).

$$(RT \ln a_{K^+} + \overline{F}^0_{K^+})_I = (RT \ln a_{K^+} + \overline{F}^0_{K^+})_{II}$$

$$(RT \ln a_{Cl^-} + \overline{F}^0_{Cl^-})_I = (RT \ln a_{Cl^-} + \overline{F}^0_{Cl^-})_{II}$$

. . . .

If we choose to express the activities in both phases in terms of the same standard state, we can write further

$$(a_{K^+})_I = (a_{K^+})_{II}$$

$$(a_{Cl^-})_I = (a_{Cl^-})_{II}$$

. . . .

Thus we are led to conclude that, on the basis of the same standard state, the activity of any ion in a soil suspension or gel is the same as in its equilibrium filtrate.* In some quarters it may be contended that this conclusion is contradicted by the well-known observation that the pH, as ordinarily determined with the hydrogen or glass electrode, may be quite different in suspensions of acid clays and in their filtrates. However, it should be pointed out that such measurements invariably involve an electrolytic bridge and a liquid–liquid junction. The magnitude of the junction potential cannot be measured. Conceivably, it may be quite different in a clay suspension and in a dilute salt solution. For these reasons, conclusions regarding H^+ activities in systems of this kind based on pH determinations are subject to question.

The activity of an ion can be expressed in terms of its concentration by means of the relationship

$$a_i = \gamma_i m_i$$

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* According to this treatment, the activity of an individual ion is defined in terms of its *electrochemical potential*. In a number of treatises (see, for example, J. N. Bronsted, *Physical Chemistry*, New York, The Chemical Publishing Company, 1938, page 276) the electrochemical potential of an ion is considered as being composed of two parts, one chemical and the other electrical: i.e., $\overline{\mu}_i = \mu_i + z_i F \psi$, where $\overline{\mu}_i$ is the electrochemical potential, μ_i the chemical potential, z_i the valency of the ion, F Faraday's member, and ψ is the electric potential of the phase. Moreover, some writers using this line of reasoning choose to define the activity of an individual ion in terms of its chemical potential, although they point out that the activity so defined has no thermodynamic significance.

where m_i is the molality of the ion and γ_i is its activity coefficient. γ_i becomes unity in an infinitely dilute solution. Many soil filtrates are dilute to the extent that the activity coefficients of their constituent ions can be taken as unity and the activities of the ions can be considered equal to their molalities. In such cases, where the total concentrations in each phase are known, it is possible to calculate relative activity coefficients for the ions in the soil suspension or gel.

When this calculation is made, it is found very often that the activity coefficient of a soil cation is very much less than unity, often of the order of 0.001. On the other hand, we find that with soil suspensions that display such Donnan effects as negative adsorption (*41*), the activity coefficient of a soil anion may be considerably greater than unity. This, however, is not an uncommon situation even with ordinary electrolytes (cf. the mean activity coefficient of 2 *M* HCl).

Ion absorption rates in soil suspensions and their filtrates

In a series of studies dealing with the "contact" and "soil solution" theories, Jenny and Overstreet (*24, 25, 37*) were able to show that with certain clay or soil suspensions containing adsorbed Na^+, K^+, or Rb^+, the rates of absorption of the ions by barley roots was greater than from the corresponding filtrates. In terms of the present argument, these experiments are rather strong evidence that the absorption rates of some soil ions are functions of their concentrations in the culture medium rather than their activities. That is, although the ion activities were the same in suspension and filtrate, the rate was greatest in the phase where the concentration was greatest.

A similar situation has been encountered in other branches of chemistry. Although most theories on reaction kinetics predict that rates will be functions of the activities of the reactants, a rather impressive number of reactions have been discovered in which the rates are determined by concentrations (*34*).

In our present state of knowledge, predications beforehand of the rate-determining factors of chemical reactions are uncertain. Also, we are probably not justified in making any generalizations concerning the absorption of soil ions. To date, the effect has been established for Na^+, K^+, and Rb^+ ions only.

Information as to whether activities or concentrations determine the absorption rates of anions is scanty indeed. Appropriate systems for testing this point would be soils that show large concentration differences of anions between the soil suspension and its filtrate. These conditions presumably would obtain in soils which show a large negative absorption of anions or in soils that adsorb phosphate, arsenate, or molybdate in large amounts. The experiments of Dean and Rubin (*11*) with a soil high in fixed phosphate would seem to indicate that the absorption rate by plants of phosphate is a function of the activity of the phosphate ion rather than of its concentration. That is, the adsorption rate from a soil high in adsorbed phosphate was apparently no greater than from its filtrate.

It is evident that a great deal more experimentation is warranted on this point. Nevertheless, the fact that the absorption rates of such important soil ions as Na^+ and K^+ are controlled by concentration rather than activities gives rise to the question as to what functions of concentrations in the soil are appropriate indications of the availability of these ions.

Relationship of ion availability to certain concentration factors in the soil

For many years attempts have been made to correlate the availability of an ion with its concentration in the exchange complex of the soil. On the whole this correlation has not proved very satisfactory.

Fairly recently, Jenny and Ayres (27) and others have attempted to correlate the availability of Na^+, K^+, and Ca^{++} with their equivalent percentage in the exchange complex of the soil (degree of saturation). In a number of cases this procedure has resulted in fairly satisfactory correlations; however, as yet the results are so limited that generalizations are not justified.

In the case of anions that are not adsorbed by soil particles, it is customarily assumed that an adequate assessment of their availability is given by their concentrations in the soil water. Whether this assumption is justified in soils that exhibit a large negative adsorption of anions has not been determined. The availability of anions such as phosphate, arsenate, and molybdate that are adsorbed in large amounts by some soils is a field for a great deal more experimentation. In the case of phosphate it begins to appear that its availability may be rather closely related to its concentration in the soil solution; that is, in a filtrate of the soil. This is additional indirect evidence that the absorption rate of the phosphate ion may be determined by its activity rather than concentration in the soil medium.

Importance of the question of reversibility of the ion absorption reaction

A knowledge of the reversibility of the absorption by roots of a given nutrient ion is of paramount importance in the estimation of its availability. This is the case because the effective rate at which a reversible reaction proceeds will be influenced by the concentrations or activities of the products as well as of the reactants. For example, if it were definitely established that the following absorption reaction were reversible

$$R' \cdot \mathrm{OH} + \mathrm{H_2PO_4^-} \longrightarrow R' \cdot \mathrm{H_2PO_4} + \mathrm{OH^-},$$

then obviously the extent to which $H_2PO_4^-$ would be absorbed would depend on the amounts of $R' \cdot H_2PO_4$ in the plant and of OH^- in the culture medium as well as on $R' \cdot OH$ and $H_2PO_4^-$. Thus, eventually it may be established that the availability of certain ions are functions of ratios of concentrations or activities.

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CHAPTER

5

Contact Phenomena Between Adsorbents and Their Significance in Plant Nutrition

HANS JENNY

Speculations on the nature of mineral uptake by roots in soils are encountered very early in botanical literature. Two states of existence of nutrients in the soil were recognized: first, nutrients in the solid portion which were considered unavailable to plants; second, nutrients dissolved in the liquid phase which could be ready assimilated by plant roots. These dissolved nutrients were known to diffuse freely in the soil moisture, and they were known also to move with the flow of water in the soil. Water containing dissolved nutrients constituted the soil solution, a fruitful concept which is still in use today. In its essence, the soil solution corresponds to the nutrient solution of the plant physiologist.

SOIL SOLUTION CONCEPT

Investigators who study the soil solution usually define it on an operational basis. The soil solution is that part of the liquid phase which can be separated from the bulk of the soil by some sort of displacement method (5), extraction or centrifugation.

To the colloid chemist it is clear that the extraction or displacement technique does not measure the adsorbed (exchangeable) cations, including hydrogen, which remain attached to the colloidal particles. As we shall see, this omission has far-reaching consequences.

In this paper we shall assign the adsorbed ions to the solid phase. Although they are surrounded by and bathed in water molecules, they are not free to diffuse in the liquid phase. The ion swarm always remains attached to the colloid particle and goes wherever it goes.

One might redefine the soil solution as comprising the dissolved electrolytes in the displaced soil extract plus the exchangeable cations and anions of the solid phase. Rather than introducing a new definition of soil solution, the writer prefers Marshall's term "ionic environment of the root" for the combination of adsorbed (exchangeable) ions and the classical soil solution ions.

Probably the most extreme proponent of a simple soil solution theory of plant nutrition was Cameron (5). As late as 1911, he stated: "There can be no doubt, therefore, that the soil solution is normally of a concentration amply sufficient to support ordinary crop plants, and is maintained at a sufficient concentration so far as mineral plant nutrients are concerned." To account for the existence of soils giving low yields, Cameron postulated and, seemingly, demonstrated that infertility of soils is caused by the presence of toxic or inhibitory organic substances in the soil solution.

Cameron labored under the erroneous impression that the liquid phase was nothing but the saturated solution of sparingly soluble soil minerals. He overlooked the contribution of the adsorbed ions to the ionic environment of the plant root.

Von Liebig, preceding Cameron by half a century, appears more farsighted. After some hesitation he repeated and confirmed Way's famous experiments on base exchange. In 1858 he expounded new ideas on plant nutrition(27):

> "It is clear that we must abandon this idea (soil solution theory) when it can be demonstrated that rain water, either alone or in conjunction with carbonic acid, does not dissolve enough of the mineral constituents to contribute significantly to plant growth. In this case the uptake of minerals must be the result of an active contributing cause residing in the root, whereby the water surrounding the root is enabled to dissolve certain mineral constituents which, otherwise, it could not do. As further consequence, the quantity of mineral substances consumed must be in proportion to the root surface of the plant and to the sum of those active mineral constituents that are contained in such portions of the soil as are in contact with the root surface."

Loosjes (*28*) hinted that Liebig foresaw the modern contact theory of plant nutrition in soils. This contention is hardly justified. In Liebig's days two essential prerequisites of the contact exchange theory were unknown; namely, the law of mass action, especially in relation to its reversibility aspects, and the concept of the electric double layer. Moreover, Liebig himself stated (p. 139): "It is very difficult to visualize in what manner plants contribute to the solubility of mineral constituents; that water is essential to their passage is obvious."

The idea that the extracted soil solution does not provide the whole answer to the question of mineral nutrition of plants in soils was sporadically advanced by several investigators, such as Comber (*7*), Kossovitch (*25*), and Truog (*44*). These attempts to attribute a more active role to the solid phase were unsuccessful for two main reasons. First, no reliable experimental material was produced which would render untenable the soil solution theory, especially if combined with the picture of carbon dioxide excretions. Second, and most important of all, no theory was advanced which provided a convincing reaction mechanism involving the solid phase.

SALIENT ASPECTS OF THE CARBON DIOXIDE THEORY OF MINERAL NUTRITION OF PLANTS

According to prevailing ideas, roots secure cations adsorbed on soil particles by means of carbonic acid exchange (Figure 1). The transfer involves the following steps:

1. Release of carbon dioxide from the root and formation of carbonic acid.
2. Diffusion of carbonic acid to the distant clay surface.
3. At the clay surface H^+ replaces K^+; the clay particle becomes acid.
4. The new ion pair, $K^+HCO_3^-$, returns to the root surface.
5. At the root surface K^+ exchanges for H^+, or $K^+HCO_3^-$ enters the root as an ion pair.

Of the last two alternatives the latter seems less probable. Overstreet, Ruben, and Broyer (*36*) immersed barley roots in a solution of potassium acid carbonate in which the anion HCO_3^- contained radioactive carbon (C^{11}). Although the roots accumulated large quantities of potassium they utilized only a small fraction, 4–5 per cent, of HCO_3^-.

Jenny and Cowan (*17*, *18*) grew soybean seedlings on pure calcium clay suspensions having an initial pH of 6.30. At harvest time the plants had removed from the clay 1.020 milliequivalents (m.e.) of calcium. The reaction of the sol dropped to pH 4.32 and the clay particles now contained 0.948 m.e. of hydrogen which they did not possess at the beginning of the experiment. While this observation is in harmony with step 3 of the carbon dioxide theory, it is also in accord with the contact theory.

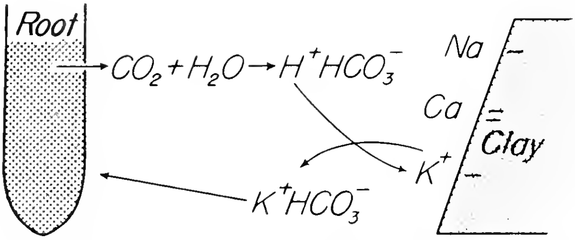

FIGURE 1. Conventional model of liberation of adsorbed ions by roots (CO_2–theory).

In this connection an experiment by Overstreet, Broyer, Isaacs, and Delwiche (*33*) is illuminating. These authors determined the uptake of cations and anions by barley roots from potassium solutions and potassium clay suspensions. They compared it with carbon dioxide evolution and the synthesis of organic acids in the cell sap of the root. The authors concluded "the excess accumulation of cations over anions is roughly balanced by organic acid anions (other than HCO_3) which are synthesized within the plant. Moreover, it is apparent that these synthesized organic acids are the ultimate sources of the hydrogen which replaces the adsorbed potassium on the clay, and not carbonic acid."

INADEQUACIES OF THE SOIL SOLUTION THEORIES OF PLANT NUTRITION

In discussing his experiments on plant growth in synthetic ion exchange media, Arnon and Grossenbacher (*3*) concluded ". . . the data do not require the invoking of a contact exchange mechanism to explain the results obtained." Allaway (*2*) expressed a similar opinion. Indeed, why should anyone wish to consider a new theory so long as the old one appears satisfactory?

A body of data exists which casts doubt on the omnipotence of the soil solution theory. We shall briefly discuss some of the more perplexing situations.

Uptake of ions by living roots from salt solutions and clay suspensions

For a period of 10–20 hours, excised, low-salt barley roots rapidly accumulate ions. It is of interest to compare the uptake of cations by roots from salt solutions and clay suspensions having equal concentrations of cations. According to Figure 2, the uptake of radioactive sodium (Na*), at higher concentrations, is decidedly greater in clay suspensions than in chloride or bicarbonate solutions (*20*). This observation is confirmed with nonradioactive sodium (*35*). In the case of ammonium clay versus ammonium chloride, the uptake of ammonium by the roots is nearly the same for the two systems. On the other hand, potassium chloride provides a better source of potassium than potassium clay. These last two experiments were conducted by Ayers (*4*) who used the Hoagland technique (*12*).

On the generally held assumption that ion uptake is a function of activities, only the potassium systems behave somewhat according to expectations. With the aid of clay membranes, Marshall (*30*) has shown that salt solutions possess higher activities than clay suspensions having concentrations of cations equal to the salt solutions. Accordingly, salt solutions should provide more efficient nutrient media. It is not known how far the experiments with barley roots can be generalized. Suffice it to emphasize that the uptake of cations from clay suspensions bears no simple relationship to ion accumulation from salt solutions having corresponding cation contents.

Significance of type of clay mineral in nutrient uptake by plants

Several years ago Elgabaly, Jenny, and Overstreet (*8*) observed that under comparable conditions barley roots accumulate more potassium and zinc from montmorillonite clay suspensions than from kaolinite clay suspensions. These observations are in striking contrast to what one would expect to happen on the basis of the carbon dioxide theory. Release of potassium and zinc from the two clay minerals by water, carbonic acid, and hydrochloric acid is greater for kaolinite than for montmorillonite. The soil solution or, more precisely, the supernatant liquid of the kaolinite suspension always contains more bases than the montmorillonite sol. Accordingly, nutrient uptake should be favored by kaolinite and not by montmorillonite as is actually observed.

Experiments performed by Mehlich and Colwell (*32*) and by Allaway (*2*) who grew cotton and soy bean plants for several weeks produced results opposite to those of Elgabaly and his co-workers. Recently, however, Elgabaly and Wicklander (*9*) confirmed the previous findings, showing that barley roots accumulate more calcium and sodium from montmorillonite systems than from kaolinite systems. We must conclude that the experiments of Elgabaly *et al.* also are valid and that for their systems and techniques the soil solution theory—with or without the carbon dioxide mechanism—is incapable of explaining the results.

Contact depletion

Living barley roots low in potassium tenaciously hold onto their content of potassium. Even intensive leaching of the roots with 380 liters of distilled water will not remove measurable amounts of potassium (*21*). Likewise, electrolyte solutions comprising sodium chloride, hydrochloric acid, sodium bicarbonate, and ammonium bicarbonate will not significantly affect the potassium status of the roots. On the other hand, as will be discussed on page 121, hydrogen clay, calcium-hydrogen clay, sodium clay, and ammonium clay very significantly deplete the root system of potassium.

More sensitive techniques involving radioactive potassium reveal similar root behavior. While some of the radioactive isotope is released by the plant to water and electrolyte solution, the outgo becomes especially pronounced when the roots are in contact with colloidal clay particles. It should be kept in mind that these experiments deal with

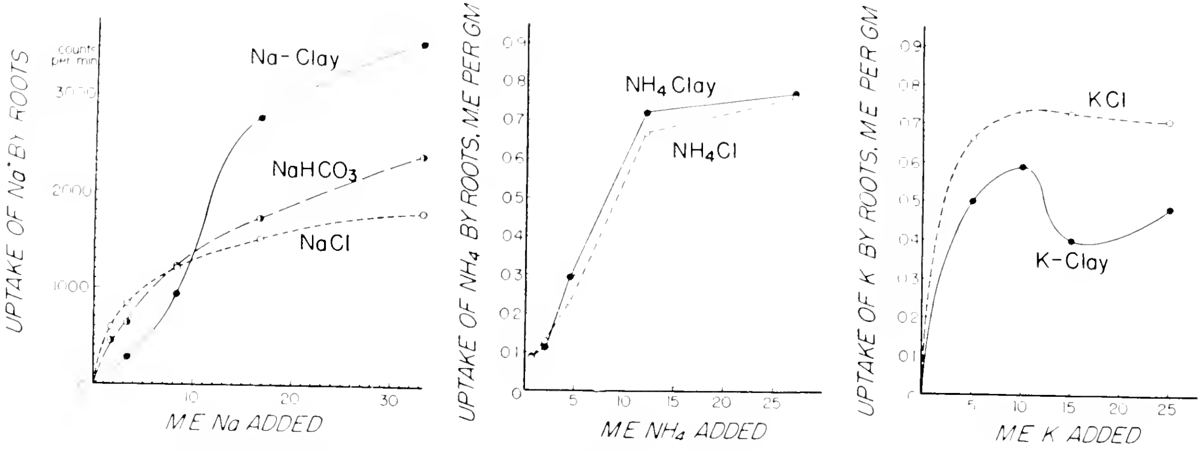

FIGURE 2. Comparison of cation uptake by roots from clay suspensions and salt solutions having equal cation content.

roots having low-salt status. High-salt roots may release copious amounts of ions to solutions.

In view of the fact that a semipermeable membrane inserted between root and clay prevents contact depletion, it appears impossible to resolve this type of root behavior in clay systems in terms of conventional soil solution theories. With Ratner (*39, 40*) we believe that desorption of root-ions by clays constitutes an important plant physiological process occurring in soils.

Uptake of radioactive columbium (Cb^{95})

Columbium added to clay behaves as an insoluble compound. At a pH value of 1.0 and at pH values commensurable with root activities, no columbium can be detected in the intermicellar liquid. Yet, as Jacobson and Overstreet (*14*) have shown, dwarf pea plants successfully compete with the clay for columbium and accumulate it in the root, and, to a small extent, in the top. These findings have been confirmed with carrots by Vlamis and Pearson.* We cannot at present comprehend the transfer of columbium on the basis of the carbon dioxide-solution theory.

Utilization of nonexchangeable potassium

Ramona loam contains 76 p.p.m. of exchangeable potassium as determined by leaching with neutral, normal ammonium acetate. Rye seedlings (Neubauer test) will assimilate, within 18 days, 264 p.p.m. of potassium from the same soil. Evidently these plants extract 188 p.p.m. of nonexchangeable potassium.

According to Peech (*38*), many investigators have postulated the following equilibria between the different forms of soil potassium:

$$\text{Nonexchangeable K} \rightleftharpoons \text{Exchangeable K} \rightleftharpoons \text{K in solution}$$

The removal of solution-potassium by the plant releases exchangeable potassium into the solution which, in consequence, causes conversion of nonexchangeable potassium to the exchangeable form. The

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*J. Vlamis, and G. A. Pearson, "Absorption of radioactive zirconium and niobium by plant roots from soils and its theoretical significance," *Science*, 111: 112–113 (1950).

practical validity of the equation in its application to the Neubauer test rests on the rate of conversion of nonexchangeable potassium.

Unpublished, exhaustive studies by D. E. Williams show that 30 days of continuous leaching of Ramona soil with various dilute acids (pH 3-6) will liberate only a fraction of the potassium extracted by the Neubauer seedlings in 18 days. Distilled, carbon dioxide-saturated water, passing through 25 grams of soil, freed of exchangeable potassium, contains about 0.10 mg. of nonexchangeable potassium per liter, apparently irrespective of the rate of flow. It is highly questionable (*13*) that plants will grow satisfactorily in a solution containing only 0.10 p.p.m. of potassium, even if the solution is continuously renewed. Roots used in the Neubauer test must be endowed with a mechanism of potassium extraction vastly more powerful than carbon dioxide leaching.

In the author's opinion, the carbon dioxide theory of liberation of adsorbed ions has generally been overrated. To use a metaphor, we have accepted its application blindly, without asking the plant how far it lives up to its tenets. Besides, the theory is unimaginative; soil water is readily enriched in carbon dioxide and little room is left for individuality of plant behavior. We must look for additional theories of plant nutrition in soils. One of these is the contact exchange theory.

THE CONTACT EXCHANGE THEORY

The contact exchange theory (*19*) describes a mechanism for reactions between adsorbents, or solids in general, without the participation of free electrolytes. It was deduced from theoretical considerations concerning the nature of ionic surfaces. Specifically, it rests on the concept of overlapping oscillation spaces of adsorbed ions, or, in another

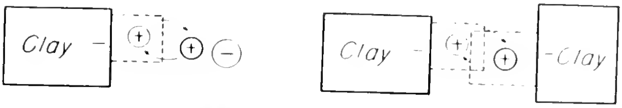
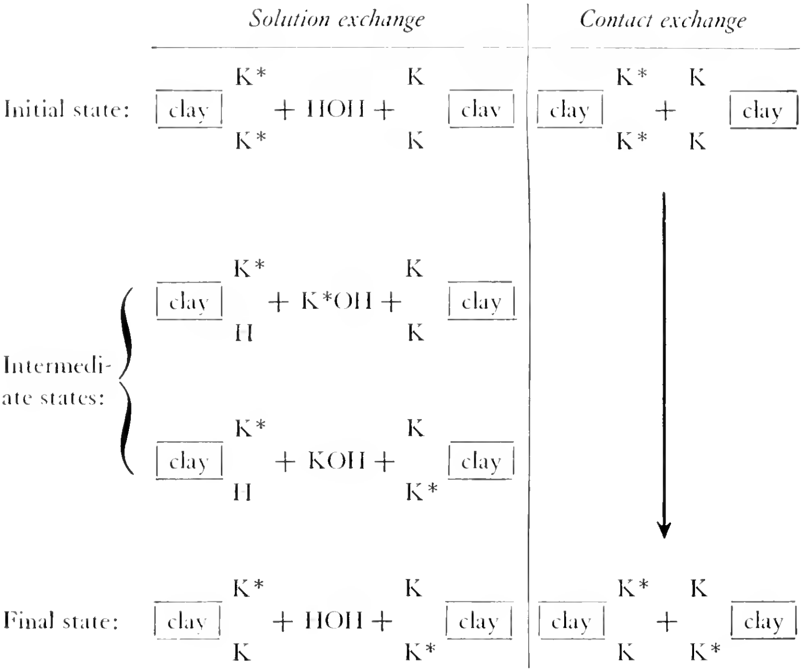


FIGURE 3. *Left:* Model of ion exchange in salt solution. *Right:* Model of contact exchange between clay particles. The dashed lines signify overlapping oscillation volumes (*15*). In these models attention is focused on individual ions.

terminology, on ion redistribution within intermingling electric double layers (Figures 3 and 4). Accordingly, the contact theory embraces all colloidal systems, or all ionic surfaces, not only soil colloids and plant roots.

To illustrate the modes of transfer of cations from one negative surface to another let us add radioactive K*-colloid to a sol containing nonradioactive potassium colloid. The two possible types of mechanisms of cation transfer occurring in the mixture may be symbolized as follows:



At equilibrium both particles contain one K*. Analysis of the initial and final states will not tell which mechanism was operative in the transfer of K* from one particle to the other. Of course, if the colloidal particles are prevented from coming close together, only the solution exchange can explain the transfer.

Whenever it is impossible to rule out experimentally one of two rival theories, a choice may be made on the basis of the intrinsic value of the theories themselves. There is one important mechanistic aspect which renders the contact theory particularly attractive.

Between negative surfaces (negative inner layers), transfer of cations may be accomplished without the aid of anions. Conversely, between positive surfaces (positive inner layers), anions may be transferred without cations. Generally speaking, an ion is transferred without an accompanying partner. Although water is usually present, it is not essential. Theoretically, contact exchange may occur in any medium, provided an electric double layer exists.

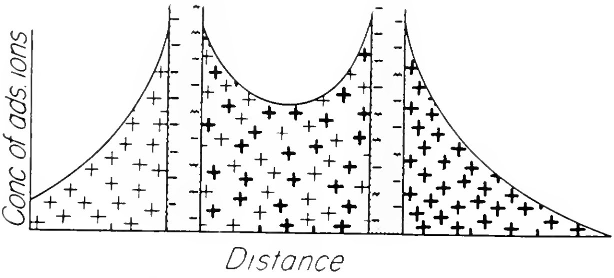


FIGURE 4. Model of contact exchange between intermingling ion swarms. Two parallel negative clay plates with undisturbed positive ion swarms on the outer surfaces and interpenetrating mixed swarms between the plates.

Sengupta (*42*) measured ammonium–sodium exchange between 2.02 m.e. ammonium colloid (Ion-X, < 0.1 mm. particle size) and 1.50 m.e. sodium colloid (Amberlite, > 0.5 mm. particle size) in 100 cc. of various media. The transfer of ammonium from Ion-X to Amberlite was 43.1 per cent in water, 45.4 per cent in methyl alcohol, and 21.7 per cent in benzene. In the latter system the rate of transfer was slow and equilibrium probably had not been attained. Ion exchange must have been primarily by contact.

In applying the idea of contact exchange to the mineral nutrition of plants in soils, it is postulated that ion swarms of the root and of the soil particles intermingle. Transfer of ions is accomplished without the aid of the soil solution. The uptake of adsorbed ions by contact is largely independent of the water content of the soil and the transpiration stream of the plant. To a certain extent, it will occur at 0° C. when metabolic activities are near a standstill.

It may even be envisaged that the entire plant is permeated by electric double layers and ion swarms, and that ions may possess locomotion in all plant parts by virtue of contact exchange and surface migration (*20*).

MORPHOLOGIC ASPECTS OF ROOT-SOIL CONTACT EFFECTS

The contact theory does not invoke the presence of carbon dioxide. Upon contact, the potassium of the clay and the hydrogen of the root directly exchange positions (Figure 5). While the contact theory dispenses with carbon dioxide it must, on the other hand, assign to the root, properties of an ionic adsorbent, that is, exchange spots. Moreover, the ions assigned to the exchange spots must oscillate sufficiently far to interact with the oscillating ions of the clay particles.

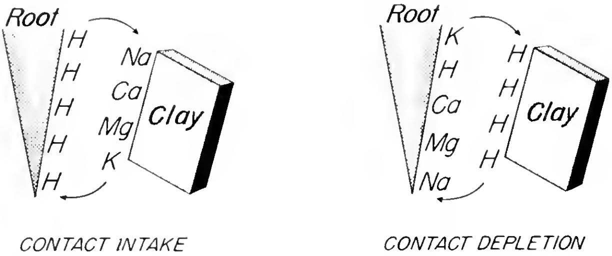


FIGURE 5. Schematic representation of contact intake and contact depletion of cations.

It is here that some plant physiologists voice their objections. Wanner (*47*) writes "Dieser Mechanismus . . . ist wenig wahrscheinlich, indem dabei die geringe Kontaktfläche zwischen Meristem und Bodenpartikel und die mit Wasser imbibierte Zellulosemembran zwischen den beiden effektiven Adsorptionsflächen nicht berücksichtigt werden."

The first objection stresses the small contact zone between meristem region and soil particles. Wanner bases his argument on the salt absorption studies of Overstreet and Jacobson (*34*) which, at 0° C., indicate preferential, strong cation absorption in the meristem region which corresponds to a band having a width of about 1 mm.* Now, one square millimeter of root surface will accommodate 10^8 clay platelets having an edge length of 100 mμ. On each of these clay particles the surface facing the root contains 6000–7000 exchangeable monovalent cations. Accordingly, in a clay soil the ion concentrations surrounding one square millimeter of root surface is tremendous and contact feeding cannot be a limiting factor in supplying ions to the root surface.

The second objection of Wanner, the existence of an inert cellulose membrane between the clay surface and the outer surface of the cytoplasm, is also voiced by Lundegårdh (*29*).

The thickness of the cellulose wall is quite variable. Lundegårdh quotes a range of thickness of 0.1–3.0μ or 1000–30,000 A. Frey-Wyssling (*10, 11*) gives a value of 0.5μ for roots. It is not inconceivable that the electric double layers of the cytoplasm and the clay particle are sufficiently diffuse to penetrate a thin cell membrane from both sides and intermingle within the intermicellar spaces. It is questionable, however, whether this direct contact exchange could bridge cellulose walls which exceed 1μ in thickness.

Considerable evidence is on hand which refutes the idea of an inert cell membrane. First of all, pure cellulose is not inactive. It has definite, though small, cation exchange properties associated with acidic groups (*43*). Second, it is very unlikely that the cellulose wall consists of pure cellulose. Frey-Wyssling in his detailed discussion of properties of cell walls reports that the cellulose membrane is permeated by intermicellar spaces which contain pectic substances, lignin, hemicellulose, and mineral substances. Of these, pectic substances are probably most abundant. They have pronounced cation exchange properties. It is also possible that active protoplasmic strands extend into some of the channels of cell membranes of growing root tips.

In the light of these considerations, the fine structure of cell walls favors contact exchange rather than disfavors it.

The barley roots used by Jenny and Overstreet have a cation exchange capacity (ammonium acetate method) of 11.0 m. e. per 100 grams of dry roots. The precise seat of the exchange spots is not known. How-

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* According to personal communication by Overstreet, at room temperature nutrient absorption is not restricted to the meristem region.

ever, if living barley roots are immersed in a positively charged iron hydroxide sol, the root surface becomes coated with positive colloids. As this reaction resembles the mutual flocculation process of oppositely charged colloids, the existence of negative charges on the root surface is strongly suggested.

Good evidence for the existence of hydrogen ion swarms surrounding the root surface is provided by the suspension effect of roots. Living roots, preferably starved of cations, are washed with carbon dioxide-saturated water (pH 4.1). If the calomel electrode of a glass electrode-pH-meter (e.g., Beckman) is gently but firmly pressed against the root mass immersed in water, the instrument records potential differences which correspond to acidities lying between pH 3–4, and often between 2–3.*

PHYSIOLOGIC ASPECTS OF ROOT-SOIL CONTACT EFFECTS

The contact theory distinguishes between contact intake and contact depletion of plant nutrients, as illustrated in Figure 5. From clays coated exclusively with potassium ions, excised barley roots sorb large amounts of potassium (Table I). On the other hand, clays devoid of potassium in the ion swarm rob the plant of potassium ions. The desorption of root ions bears no simple relationship to pH, and it is not the result of root injury. Needless to say, it cannot be detected if root and clay are separated by a semipermeable membrane (*21*). There appears to exist for each type of ion on the soil colloid a critical degree of saturation at which the root neither gains nor loses the ion in question. This null-point would depend on the amount and nature of the clay and on the physiological condition of the plant. As the critical degree of saturation is being approached, the plant suffers severe metabolic disturbances such as the rosette disease in lettuce (*46*) which is conditioned by calcium starvation.

Ratner (*39, 40*) in two papers published in English reported strik-

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*D. E. Williams, and N. T. Coleman, "Cation exchange properties of plant root surfaces," *Plant and Soil*, 2:243–256 (1950). In this connection the following paper should be consulted: H. Jenny, T. R. Nielsen, N. T. Coleman, and D. E. Williams, "Concerning the measurement of pH, ion activities and membrane potentials in colloidal systems," *Science*, 112:164–167 (1950).

ing instances of depletion of ions from barley plants at different stages of growth. Loss of cations to clays containing 30 per cent of calcium and 70 per cent of sodium was not restricted to roots; it extended to stalks, leaves, and even to ears.

While loss of nutrients to clays is an observation of long standing (*1, 18, 23*), it had never been systematically explained. Now, in the light of the colloid interaction theory it reveals itself as the legitimate partner of contact sorption.

TABLE I

Gains and Losses of Potassium by Barley Roots in Various Clay Systems

Ions on Clays, milliequivalents in 3 liters of suspension							Increase or Decrease of K Content of Roots	Final pH of Suspension
K	H	Na	NH_4	Ca	Total	D*		
25	0	0	0	0	25	100%	+44.7%	6.48
5	20	0	0	0	25	20	+28.5	4.40
0	30	0	0	0	30	0	−66.0	3.52
0	0	9.9	0	0	9.9	0	−13.2	4.70
0	0	0	9.9	0	9.9	0	−32.4	5.14
0	0	0	0	6.8	6.8	0	−5.8	6.43
Hydrochloric acid							−5.1%	4.10

D* = degree of potassium saturation

In presence of clay strongly pronounced counter migration of cations can be demonstrated. From potassium-hydrogen clays (pH 4.4) having not too low degrees of potassium saturation, barley roots accumulate potassium and increase their potassium content by 28.5 per cent. At the same time the roots lose calcium ions, to the extent of 22.2 per cent. From calcium-hydrogen clays (pH 5.25), roots take up calcium ions (6.4 per cent), but experience a loss of potassium ions amounting to 19.4 per cent. With radioactive indicators it is possible to prove that a given ion species simultaneously moves into the root and out of the root. We must think, therefore, in terms of net accumulation and net depletion.

EXAMINATION OF IONIC ENVIRONMENT OF ROOTS AND THE MEASURING OF CONTACT UPTAKE

In a soil pore filled with water the liquid phase contains dissolved electrolyte (H_2CO_3, $Ca(NO_3)_2$, K_2SO_4, etc.) and, near the surface of the solid phase, the swarm of adsorbed ions. Figure 6 schematically depicts a root segment—stripped of its electrical properties for the simplification of drawing—immersed in a region consisting of a potassium ion swarm and dissolved potassium chloride. According to the contact theory both forms of potassium are available to the plant, in fact, the root would not be able to distinguish between the two potassium species. The classic soil solution theory, on the other hand, would not recognize the potassium of the ion swarm as a direct source of potassium. As Mattson (*31*) recently put it: the plants feed directly upon X_K (K of the free salt) and only indirectly upon Z_K (K in combination with clay); the latter must be replaced by another cation before it is made available.

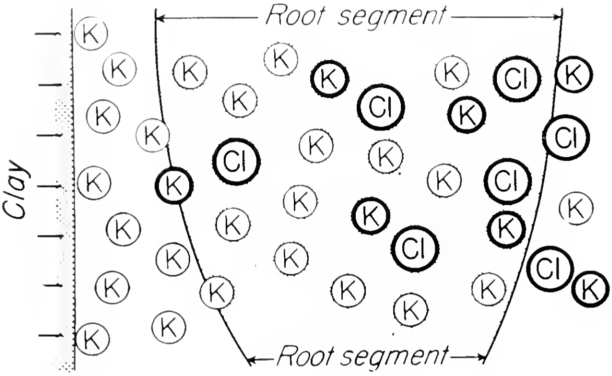

FIGURE 6. Root segment immersed in an ionic environment consisting of ion swarm K (light circles) and KCl (heavy circles). The ions are meant to be on the outside of the root segment.

To experimentally ascertain the reactivity of adsorbed potassium, it is necessary to separate the potassium chloride solution from the ion swarm by centrifugation or ultrafiltration. If root behavior in the original, mixed system differs from the root behavior in the extract, a contact effect is strongly suggested.

The required extraction of the "intermicellar liquid" is often difficult to achieve. Seemingly clear ultrafiltrates, obtained in high-speed centrifuges, may reveal numerous fine colloidal particles if viewed in the ultramicroscope. In addition, there are certain aspects connected with Donnan equilibria which must be taken into consideration. The situation is further complicated by carbon dioxide evolution of the roots. Prior to separation the system has to be saturated with carbon dioxide to simulate conditions of root environments.

TABLE II

Uptake of Radioactive Rubidium by Barley Roots from Rb-H-Clays and Corresponding Supernatant Liquids (*20*)

Concentration of Sol	Degree of Rb Saturation	Intake of Rb*, Expressed as Counts per Minute: From clay suspension	Intake of Rb*, Expressed as Counts per Minute: From bicarbonate solution	Increase of Clay Sol over Carbon Dioxide Extract
4.94%	1.0%	18.5	6	208%
2.47	2.0	24.5	12.5	96
1.65	3.0	27.5	20	38
0.99	5.0	30	30	0

The soil solution theory contends that any ion removed from the soil solution by plants is replenished by the solid phase. Some investigators even assume that the composition of the soil solution remains constant. Since rate studies also require constant composition of media, the extracted intermicellar liquid must be offered to the roots in such large amounts that the contents remain unchanged during the absorption process.

Only two experiments have been published (*20, 35*) which satisfy all of these requirements: the sorption of radioactive rubidium as a function of the degree of saturation, and the sorption of radioactive sodium from various clay concentrations. Table II reports the uptake of radioactive rubidium from rubidium-hydrogen clay suspensions and rubidium bicarbonate solutions, the latter having the same rubidium concentration as the carbon dioxide-saturated supernatant liquid of the rubidium-hydrogen sol. In spite of possible contact depletion by hydrogen clay, the roots secure more rubidium from the clay sol than from the artificial intermicellar liquid. As roots were in contact with solution and suspension for only one second, ion accumulation inside the root was largely excluded. We are probably dealing here with an exchange involving outer root surfaces only.

Figure 7 portrays the sorption of radioactive sodium by groups of 21 intact barley plants during a period of 10 minutes as a function of sodium clay concentration. The upper curve shows the uptake of sodium from a large volume of sodium clay suspension. The lower curve indicates the sodium sorption from a large volume of the corresponding intermicellar liquid, saturated with carbon dioxide prior to separation. During the process of ion accumulation by roots, clay sols, as well as sodium bicarbonate solutions, were continually aerated. In our opinion, the difference in the two curves demonstrates direct utilization by roots of adsorbed sodium.

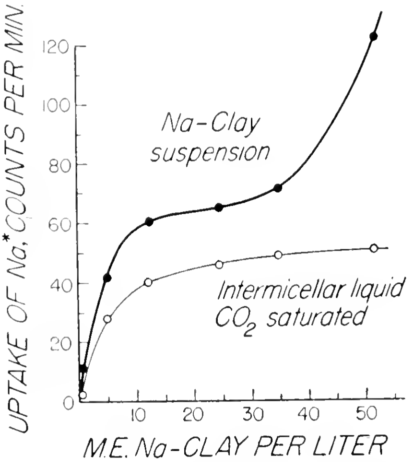

FIGURE 7. Demonstration of direct utilization of adsorbed ions by roots.

EQUILIBRIUM STUDIES ON ION EXCHANGE BETWEEN ADSORBENTS

Symbolically, we may formulate the relationship of contact intake and contact depletion between plant roots (R) and clay particles:

$$\overset{R}{\bigvee} \text{H} + \text{K}\,\boxed{\text{clay}} \rightleftharpoons \overset{R}{\bigvee} \text{K} + \text{H}\,\boxed{\text{clay}}$$

The question whether the reaction will tend to the left (contact depletion) or to the right (contact uptake) depends on the amounts of the two adsorbents and on the forces with which the various ions are held to the two surfaces.

In approaching a quantitative elucidation of the above process, it appears advisable to first study in detail the exchange reaction between two adsorbents in vitro. Let us consider the exchange of the ammonium and sodium ions, between two different adsorbents, either by solution or by contact, as represented by the following system:

$$\boxed{\text{Ion-X}}\,\text{NH}_4 + \text{Na}\,\boxed{\text{Amberlite}} \rightleftharpoons \boxed{\text{Ion-X}}\,\text{Na} + \text{NH}_4\,\boxed{\text{Amberlite}}$$

Amberlite and Ion-X are artificial cation exchange resins. It is possible to study the reaction quantitatively by mixing coarse Amberlite particles ($>$ 0.5 mm.) with fine Ion-X particles ($<$ 0.1 mm.). After a shaking period of several days the two adsorbents are separated by wet sieving and analyzed individually. In the writer's laboratory, Dr. K. Sengupta has shown that the above reaction gives a characteristic exchange constant according to the equation:

$$K = \frac{\text{Na}\,\boxed{\text{Ion-X}} \cdot \text{NH}_4\,\boxed{\text{Amberlite}}}{\text{NH}_4\,\boxed{\text{Ion-X}} \cdot \text{Na}\,\boxed{\text{Amberlite}}} = 1.46 \text{ (experimental)}$$

It is possible to calculate this exchange constant without actually mixing the adsorbents, provided the ordinary base exchange reactions between the adsorbents and neutral salt solutions are known.

Consider the two connecting vessels of Figure 8. Initially the stopcock is closed. The left hand vessel (system X) contains coarse ammonium–Ion-X and sodium chloride. At equilibrium we have:

$$\boxed{\text{Ion-X}}\ \text{NH}_4 + \text{NaCl} \rightleftharpoons \boxed{\text{Ion-X}}\ \text{Na} + \text{NH}_4\text{Cl}, \text{ or}$$

$$\frac{\text{Na}\ \boxed{\text{Ion-X}}}{\text{NH}_4\ \boxed{\text{Ion-X}}} \cdot \frac{(\text{NH}_4)_X}{(\text{Na})_X} = k_X = 0.793 \text{ (experimental)}$$

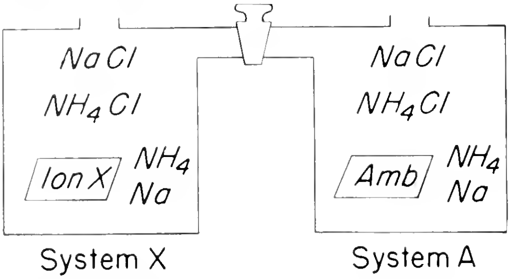

FIGURE 8. Device for deriving exchange equations between adsorbents.

This equation holds for wide variations in the amounts of sodium chloride or adsorbent used.

The right-hand vessel (system A) contains coarse ammonium-Amberlite and sodium chloride. The equilibrium equation assumes the form:

$$\frac{\text{Na}\ \boxed{\text{Amberlite}}}{\text{NH}_4\ \boxed{\text{Amberlite}}} \cdot \frac{(\text{NH}_4)_A}{(\text{Na})_A} = k_A = 0.532 \text{ (experimental)}$$

Again variations in the amounts of reacting substances do not substantially alter k_A.

Now we open the stopcock and let a new equilibrium be established. Whatever the initial salt concentrations were, we have now:

$$(\text{NH}_4\text{Cl})_X = (\text{NH}_4\text{Cl})_A \text{ and } (\text{NaCl})_X = (\text{NaCl})_A$$

By mere algebraic rearrangement we obtain:

$$K = \frac{\text{Na}\ \boxed{\text{Ion-X}}}{\text{NH}_4\ \boxed{\text{Ion-X}}} \cdot \frac{\text{NH}_4\ \boxed{\text{Amberlite}}}{\text{Na}\ \boxed{\text{Amberlite}}} = \frac{k_X}{k_A} = \frac{0.793}{0.532} = 1.49, \text{ Q.E.D.}$$

In terms of the oscillation volume theory (*15*), the constant K comprises four oscillation volumes, conditioned by the two different ions each being held on two different adsorbents. For the system under consideration:

$$K = \left[\frac{V_{NH4}}{V_{Na}}\right]_X \cdot \left[\frac{V_{Na}}{V_{NH4}}\right]_A$$

For interacting mono-divalent ions we write:

$$\boxed{\text{Ion-X}}\begin{matrix}NH_4\\NH_4\end{matrix} + Ca\,\boxed{\text{Amberlite}} \rightleftharpoons \boxed{\text{Ion-X}}\,Ca + \begin{matrix}NH_4\\NH_4\end{matrix}\boxed{\text{Amberlite}}$$

Applying the base exchange equations of Krishnamoorthy, *et al.* (*26*), the following equilibrium equation may be derived:

$$\frac{Ca(1\tfrac{1}{2}\,Ca + NH_4)\,\boxed{\text{Ion-X}}}{\left(NH_4\,\boxed{\text{Ion-X}}\right)^2} \cdot \frac{\left(NH_4\,\boxed{\text{Amberlite}}\right)^2}{Ca(1\tfrac{1}{2}\,Ca + NH_4)\,\boxed{\text{Amberlite}}} = \frac{k_A}{k_X} = k$$

The symbol k_A represents the equilibrium constant for the system Ca-Amberlite + 2 NH_4Cl and k_X the constant for Ca-Ion-X + 2 NH_4Cl.

In numerous experiments Sengupta has shown that the exchange of ions of equal valency (NH_4–Na, NH_4–K, Ba–Ca) between two adsorbents is normal, that is, the observed equilibrium constant is identical, within experimental error, with the calculated constant (Table III). The ammonium-cesium pair possibly constitutes an exception.

This identity does not exist for ion pairs of unequal valency (Na–Ca, NH_4–Ca). If large particles of ammonium-Amberlite are mixed with small particles of calcium-Amberlite (same type of adsorbent), the exchange constant should be 1, but according to Table III it is over twice as large—namely, 2.11. In every case listed in Table III, the observed exchange constant differs profoundly from the calculated one.

Interestingly enough, the abnormality disappears when free salts are added to the adsorbent mixtures. Now, the observed exchange constant agrees with the calculated one. At present we do not know whether this peculiar behavior of the mono-divalent ions is the result of experimental technique or of colloid interaction.

TABLE III

Exchange Constants Between Adsorbents (K. Sengupta)

Adsorbent I		Adsorbent II		*K* Obtained	*K* Calculated
Type	Particle size	Type	Particle size		
NH_4-Ion-X	>0.5 mm.	Na-Ion-X	$<1.5\ \mu$	1.04	1.00
Ca-Ion-X	>0.5 mm.	Ba-Ion-X	$<1.5\ \mu$	0.99	1.00
Na-Amberlite	>0.5 mm.	NH_4-Ion-X	<0.1 mm.	1.46	1.51
Na-Amberlite	>0.5 mm.	NH_4-bentonite	$<0.1>$ 0.05 mm.	0.40	0.39
NH_4-Ion-X	>0.5 mm.	Cs-bentonite	$<1\ \mu$	0.134	0.105
NH_4-Amberlite	>0.5 mm.	Ca-Amberlite	$<1.5\ \mu$	2.11	1.00
Na-Amberlite	>0.5 mm.	Ca-Amberlite	$<1.5\ \mu$	2.30	1.00
NH_4-Ion-X	$<1.5\ \mu$	Ca-Ion-X	>0.5 mm.	0.415	1.00
NH_4-Amberlite	>0.5 mm.	Ca-bentonite	$<1\ \mu$	18.70	6.92
NH_4-Ion-X	<0.1 mm.	Ca-Amberlite	>0.5 mm.	1.91	3.00

TIME RATES OF ION TRANSFER BETWEEN ADSORBENTS

The aforementioned adsorbent-adsorbent studies deal with equilibrium states. In so far as alkali metal ions are concerned, the transfer may be visualized as taking place through solution or by contact. In either case, the amount of net transfer is the same. It is, however, highly probable that the rate of transfer is greatly accelerated by contact exchange. In fact, conditions exist under which solution transfer is so slow that for practical purposes it is nonexistent. Good illustrations are provided by the behavior of divalent iron adsorbed on bentonite (*20*). More recent experiments furnish information on columbium and nonexchangeable potassium.

Experiments with columbium (K. Sengupta)

Radioactive columbium, Cb^{95}, carrier-free and dissolved in 3 *N* sulfuric acid, was added to Amberlite and to clay suspensions. After excess acid had been washed out the supernatant liquid was completely free of columbium. A 0.17 per cent suspension of columbium-hydrogen clay had a pH of 3.0. Adding hydrochloric acid to bring the suspension to 0.025 *N* hydrochloric acid did not release any columbium; neither did sodium chloride, calcium chloride (0.08 *N*), or calcium hydroxide. According to Klimenko and Syrokomskii (*24*), columbium precipitates above pH 0.4.

Whereas dilute acids and neutral salts are unable to release columbium, contact will do so. The transfer is reversible; columbium will move from fine clay to coarse Amberlite, and vice versa. During four days of shaking of adsorbent mixtures the following amounts of columbium were transferred from left to right:

Cb-H-bentonite + H-Amberlite	8.6 per cent
Cb-Na-bentonite + Na-Amberlite	5.8 per cent
Cb-Ca-bentonite + Ca-Amberlite	1.7 per cent
Cb-Na-Amberlite + Na-bentonite	6.0 per cent
Cb-Rb-Amberlite + Rb-bentonite	10.4 per cent

Time curves indicate that in these experiments equilibrium had not yet been reached. Although the rate of transfer is slow, it is in the realm of practical significance.

Release of nonexchangeable potassium

D. E. Williams prepared mixtures of Ramona soil and Ion-X adsorbent. After one week of shaking the mixtures were leached with ammonium acetate and potassium was determined in the leachate. According to Table IV much larger amounts of nonexchangeable potassium are liberated by contact with adsorbents than by leaching with the corresponding neutral salts. The liberation of fixed potassium becomes especially noticeable when hydrogen-Ion-X or hydrogen-Amberlites are used. Conceivably the hydrogen ion swarm surrounding the root likewise acts as an efficient releaser of nonexchangeable potassium.

It appears plausible that the contact effects involving columbium and nonexchangeable potassium explain, partially at least, the root behavior discussed on pages 114 and 115.

EXTENT OF CONTACT EXCHANGE IN SOILS

In any soil both solution and contact mechanisms will be operating. As far as macronutrient cations are concerned, the soil solution mechanism would be expected to predominate in sandy soils, whereas in clay soils contact would be the decisive factor. Also, the lower the salt content of the soil solution the greater will be the contribution of contact exchange. For those micronutrient cations, including iron, which are largely insoluble at higher pH values, contact exchange may well be the dominant mode of acquisition by roots.

TABLE IV

Extraction of Nonexchangeable Potassium from Ramona Soil

Extractant and Method	Nonexchangeable Potassium Extracted, p.p.m.
Neubauer rye seedlings test, 18 days	188
Leaching with *N* NH_4-acetate, 8.5 liters in 35 days	0
Leaching with CO_2 saturated water (pH 4.0)	
8.2 liters in 35 days	15
10.0 liters in 14 days	27
Leaching with *N*/10 NaCl, 1.25 liters in 7 days	23
Shaking 5 g. of soil for 7 days with 25 m.e. NH_4-Ion-X, pH = 7.6	68–114
Shaking 5 g. of soil for 7 days with 25 m.e. Na-Ion-X, pH = 7.8	110–152

CONCLUSION

The contact theory of mineral nutrition of plants in soils is only a theory. If the scientist-philosopher Campbell (6) is right, we shall never know whether or not it portrays the truth, for as he says, theories can never be proven, they can only be disproven.

However, the contact theory is a productive and stimulating theory. It assigns to the root surface the role of active, individualistic, genetic, and physiologically conditioned participation in the liberation of adsorbed nutrient ions. It enables the plant to feed directly upon the solid phase. It insists upon the interplay of soil colloids and plant colloids.* In short, it discloses new vistas on the behavior of plants in soils.

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*As Dr. Norman points out elsewhere in this volume, contact exchange should likewise include the surfaces of the microbial soil population.

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CHAPTER

6

The Effect of Soil Physical Properties on Nutrient Availability

J. B. PAGE and G. B. BODMAN

Since the beginnings of plant science the major emphasis has been placed upon the chemical properties of the soil and plant nutrients. Liebig stated in 1840: "The crops on a field diminish or increase in exact proportion to the diminution or increase of the mineral substances conveyed to it in manures." This view has been widely accepted, and can easily be understood in light of the very favorable yield increases usually obtained when fertilizers are applied to the soil. The proper use of fertilizers has become an accepted and necessary part of farm operations. There are, however, other factors of importance in crop production. This has long been recognized but comparatively little attention has been paid to the physical properties of the soil, particularly as they affect plant growth and fertilizer response. Studies in soil fertility and soil physics have proceeded concurrently with but little appreciation of the significance of the interaction of soil physical properties upon the better understood and more intensely studied chemical properties of the soil. Many accept the physical properties of a soil as being extremely important but apparently feel that little or nothing can be done to effect changes or improvements in such properties. With some soils, particularly the well-drained sandy soils, such a view may be partly justified since they have a textural porosity which permits favorable water and air movement. For a great many very important agricultural soils, however, the physical behavior is dependent upon structure and aggregation and these not only play a very important role in crop production but are subject to rapid change with time or treatment. There appears to be little appreciation of this fact. This is indicated in part by the very common tendency to depend upon fertilizers alone with little thought given to physical factors when it is desired to increase the productive capacity of a soil.

In recent years there has been an increasing appreciation on the part of some workers of the importance of the physical properties of the soil in crop production. The reasons for this are probably threefold: (*a*) with general adoption of high yielding varieties of crops and heavy fertilization rates, frequent failures to obtain the expected yield increases have shown that factors other than fertility often limit yields; (*b*) with continued cultivation many of our soils have lost or are losing much of their organic matter with a corresponding deterioration of favorable soil structure, so that physical properties are becoming poor enough to seriously limit plant growth, even though fertilizers may be liberally supplied; and (*c*) under semiarid and arid conditions, where initial content of soil organic content was low, a change from native vegetation growing on undisturbed soil under low rainfall to introduced field and orchard crops growing on frequently tilled, irrigated soil, has been observed to be associated with distinct structural changes.

Recent well-publicized attempts to make record-breaking corn yields have shown very strikingly that for high yields to be obtained, the physical, as well as the chemical factors must be at their optimum. It has also become quite apparent that unfavorable physical properties have profound effects upon the chemistry of the soil and upon availability of plant nutrients, both native and added. Little direct work has been done on this subject, so that there is need for a general appreciation of this lack in our knowledge of the soil-plant system, and for a concerted attack on the problems by both soil and plant scientists. It is the purpose of this paper to point out the importance of the physical properties in crop production, the ways in which physical properties change or affect nutrient availability, and the need for a general realization of the inadequacy of the strictly chemical approach and for the study of the soil as a system.

SOME FACTORS ESSENTIAL FOR PLANT GROWTH

The soil factors which are essential for normal plant growth are: (*a*) a favorable soil reaction and an adequate supply of all the essential nutrients, (*b*) a favorable supply of water, (*c*) adequate oxygen, (*d*) favorable temperature, and (*e*) friability or looseness of the soil so that roots are not restricted in their free growth and development. Of these factors it is usually assumed that the supply of nutrients and the reaction can be controlled through addition of fertilizers and lime, but where the other factors are unfavorable, added nutrients may not be effective and hence, in the broad sense of the term, can be considered as being unavailable. The role of the water supply in affecting availability of nutrients will be the topic of another paper in this symposium and will not be discussed here.

In discussing the remaining topics, "availability" will be treated in the sense indicated above, i.e., whenever plants fail to assimilate and utilize nutrients, the nutrients will be considered as being unavailable even though they may be in solution or appear to be readily available from chemical tests. It is realized that this is an unorthodox usage of the term "availability" but, as will be seen later, the effect of physical properties of the soil on plant growth and utilization of nutrients may be very significant. In the opinion of the authors too much reliance on a strictly chemical definition of availability of plant nutrients is misleading and only through adoption of the broader definition and all that it implies can we gain a proper understanding and interpretation of plant growth and fertilizer response.

When soil physical properties are unfavorable, nutrient availability may be reduced in any or all of the following ways: by restriction of root extension, thus limiting the volume of the soil which the roots can penetrate and the number of particles with which root contact can be made; by affecting the chemistry of the soil so that nutrients are no longer in soluble form; by limiting the metabolic activity of roots so that they can no longer function properly in assimilating the soluble or exchangeable nutrients in the soil.

SOIL PHYSICAL PROPERTIES

The physical properties of a soil play a significant role in plant growth by controlling the air, water, and, to a certain extent, nutrient supply to the roots of growing plants. In considering these properties we are primarily concerned with the pore spaces between the soil particles because it is here that roots exist, and the necessary water and air movements occur. The proportion of the total soil volume made up of pore space, the size, and shape of the pores are all extremely important. These characteristics in turn are governed by two properties: the particle-size distribution and the arrangement of the soil particles, particularly as they may be clustered into larger aggregates or crumbs.

Particle-size distribution and dependent properties

That quality of a given sample of soil taken from the soil profile, which may be expressed as a particle-size distribution curve, probably represents the closest approach to an intrinsic physical soil property. But it must be understood that in order to obtain reproducible results particle-size measurements must be made according to standardized arbitrary methods of soil pretreatment and dispersion.

For purposes of obtaining expressions of "ultimate" particle-size distribution, dispersion methods commonly in use aim at the highest possible dispersion short of actual solution. Expressions having this basis are now used in the choice of soil texture names. However, C. L. Clark (29) pointed out in 1933 that at that time there was no proof in support of the correctness of the assumption that a unique particle-size distribution exists for a given soil sample and, to the authors' knowledge, none has been presented in the subsequent fifteen years.

From the published experiences of investigators in soil physics it must be concluded that most, if not all, other physical properties that have been examined are remarkably susceptible to modification by external factors, and to external forces of one kind or another which have been brought to bear upon the soil. The physical factors which affect plant-soil interrelations, therefore, are—with the qualified exception of particle size of the solid phase—notably subject to quantitative change, brought about both by natural events and by the practice of soil management. Under field conditions, alterations associated with the passage of time appear to proceed least rapidly with the property of particle-size distribution.

It is doubtful that any physical property of soil remains uninfluenced in some respect by the proportions which the soil contains of particles of different sizes, since the "ultimate dispersion" obtainable for a given soil (by the arbitrary methods already mentioned) is itself a manifestation of the particle-size distribution, and the extent to which this limiting degree of dispersion is approached is of great significance. Cultivation and management practices, the growing plant, microorganisms and the weather, all tend to shift the degree of dispersion of the soil as displayed at any one instant closer to, or further away from, the state of ultimate dispersion.

The physical properties to be discussed may all be shown, under certain conditions, to be functions of the degree of dispersion of the solid phase. Yet, at present, nothing less than a complete distribution curve and a fundamental expression of aggregation status, together with full information concerning its chemistry, mineralogy, microbiology, and organic matter, will even approximately specify the physical and physico-chemical behavior of the soil under a given sequence of treatments and changes in water content. The functional relationships are extremely complex.

Role of aggregation

The tendency of soil particles to form clusters or aggregates which resist dispersive treatment has received much attention by soil physicists. Measurements of approximate aggregate size distribution by sieving in air, and sieving, shaking, settling, or elutriating in water, have been used to provide numerical expressions of soil structure. Although the results are affected by the method of determination used and no standard method of measurement has yet been adopted, aggregate size distributions have been shown to affect other physical soil properties and also plant growth.

Clarke and Marshall (*30, 31*) and others have shown the existence of a significant positive effect of clay on water-stable aggregation. They found that a significant decrease in water-stable aggregation* resulted

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*Aggregation was expressed in terms of the amounts of primary particles smaller than a given size which were present in aggregates larger than that size.

from increasing periods of cultivation of South Australian grassland in the red-brown earth zone. The major part of the decline took place during the first five years out of a total of twenty years of cultivation. Similar observations have been made in this country.

The effect of aggregate size upon yield of *Synapis alba* (white mustard) grown in Mitscherlich pots filled with soil aggregates of different size classes was studied by Schuylenborgh (*62*). The aggregates and crumbs were obtained by screening a cloddy clay soil. With respect to structural state and aggregate size class, Schuylenborgh found that dry weight yields increased in the order: (compact structure) $<$ (4–5 mm.) $<$ (2–4 mm.) $<$ (finer than 1 mm.) $<$ (1–2 mm.) $<$ (crumb structure).* These results are in general agreement with those obtained by Kwasnikov (see Krause, *45*) who conducted a somewhat similar experiment. Doyarenko (see *45*) and Schuylenborgh (*62*), respectively, regard the aggregate size classes 1–2 mm. and finer than 1 mm. as highly desirable. Unfortunately, the last category fails to specify the amount of aggregation within this size class. It cannot be said that any one aggregate size class is best for all soils which possess the tendency to form stable aggregates. It seems more likely for soils of different intrinsic physical and chemical properties that different states of aggregation will be required for the growing plant. In this respect, moreover, different plants may have different requirements.

The crumb structure is less permanent than that associated with the water-stable aggregates of size classes $<$ 1 mm. and 1–2 mm. For many soils in certain climates it may be too costly to attempt to create this structural state. Superior yields on the crumb structure and finer aggregates were attributed by Schuylenborgh to the greater surface areas there exposed for root exploration. This may be the explanation for the results obtained with this particular soil but, omitting the crumb structure, there is no evidence to indicate that some other size classes than those finer than 2 mm. might not be superior for other soils, e.g., those which form soft aggregates.

The influences of particle size upon the availability to plants of soil

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*Crumbs have weak cohesion and lack distinct dimensions and shape; they possess a honeycomb structure formed by the combination of primary soil particles with larger aggregates (Schuylenborgh).

water may be demonstrated, for soils of different texture, by direct measurement of the wilting point (*72*) or from moisture potential-moisture content curves, which may be obtained by means of pressure membrane apparatus (*58*) and by other methods (*72*).

The permanent wilting percentage and moisture equivalent (a function of particle-size distribution) values published for a number of different surface soils by Richards and Weaver (*57*) and by Veihmeyer and Hendrickson (*69*) indicate a high positive correlation. This may be explained by the existence, within soils of high clay content, of very fine pores that retain water against plant withdrawal forces and that are present in greater numbers in the clay-rich soils than in those of lower clay content. Although the functional relationship between permanent wilting percentage and soil is far from a simple one and may involve mineralogical and physical characteristics, the proportion of very fine particles present is possibly the most important single factor affecting this property in nonsaline soils. For a given total water content below the wilting point the energy of water retention generally increases considerably with increasing fineness of texture.

Many fine-textured highly water-retentive soils also show a marked tendency to be very plastic and to remain wet for a comparatively long time. Such soils are too frequently worked while they are still wet, with the result that puddling occurs and the soils thus become even less satisfactory for plant growth. Strikingly harmful effects on plant growth have been observed both in the field and the laboratory as the result of soil puddling: the process of mechanically working a soil and altering its structure so that, by dispersion of the aggregated particles, the coarser pores are destroyed.

For a definite expenditure of mechanical work of compression and shear, the amount of dispersion of aggregates by puddling treatment and the resultant diminution in apparent specific volume appear to depend upon the moisture status of the soil, the amount of mechanical work already expended in reducing the apparent specific volume, and the particle-size distribution (*13*). This last, of course, within the limitations stated earlier, represents the ultimate in mechanical dispersion for a given soil.

It has been shown by Thomas (*67*), Day (*32*), and Buehrer and

Rose (*19*) that, at a given moisture content and between certain moisture potential limits, dispersion by puddling diminishes the moisture potential, i.e., moisture retention forces are increased. Although the diminution appears significant, the magnitude of the effect in some cases may be slight (*12*). McGeorge and Breazeale (*51*) expressed the opinion that puddling increased the moisture content at which plants first displayed symptoms of moisture unavailability.

Total porosity diminution by puddling proceeds at the expense of the coarser pores, which are destroyed relatively easily. It is largely in these coarser pores that air and water movement occur and that roots find their most favorable environment. The benefit derived from aggregation is largely associated with the increase in the important larger pore spaces in the soil. Sands, of course, may have sufficiently large pores for normal air and water movement but the pore spaces in most silts and clays, if these soils are not aggregated, are usually so small that capillary forces cause them to remain full of water to the exclusion of air. The persistence of smaller pores, despite puddling treatment, can be explained in three ways, according to Rubin (*59*): (*a*) destroyed small pores of a given radius are replaced by others resulting from decrease in radius of larger pores; (*b*) smallest pores are filled with water which resists removal and prevents pore collapse; and (*c*) small pores owe their existence, in large part, to a more stable particle arrangement in their immediate vicinity, i.e., they may (and probably do) exist in greater abundance within the real or potential aggregates which are present in the soil mass.

Increase in relative abundance of very fine pores conceivably may affect water availability to plants, directly, by increasing the energy of water retention and, indirectly: (*a*) by offering greater resistance to root penetration, (*b*) by decreasing the supply of necessary oxygen to the roots with the result that roots no longer function normally in absorbing water, and (*c*) by increasing, over short distances, the resistance to water movement toward the plant roots. The mechanisms involved should be given more attention than they have heretofore received, but the net effect to the plant would be qualitatively similar, namely, the availability of the existing water supply would be diminished.

Penetrability by roots

The impenetrability of unfractured, dense rock to plant roots needs no comment. In shallow residual soils plant growth may be seriously restricted by the presence of continuous rock masses. Dense layers of soil also may be expected to resist root penetration provided that the density is sufficiently high or the pores sufficiently small. For example, restricted root growth may be observed over the surface of puddled cultivation-soles in citrus orchards and within crevices over the surfaces of dense structural columns in the subsoils of some alfalfa fields. Uptake of water and other nutrients will thus be restricted. In California there is now being followed a practice of noncultivation of orchards whereby danger of puddling is diminished and previously puddled soils are allowed, by natural processes of wetting and drying in the undisturbed state, to recover their former more open structure. Root development is thus enhanced.

Soils of low total porosity and relatively high microporosity may be almost impermeable to water. The B horizon of the San Joaquin series (California) has been shown (*11*) in some profiles to have a total porosity (0.26 to 0.34) inadequate to accommodate its moisture equivalent water, the magnitude of the latter being determined in the standard way on disturbed and crushed soil lumps. This horizon, assuming its penetrability to both roots and water, would be deficient in the total water available for plant growth if for no other reason than that the upper limit of growth water would be significantly less than in the case of the same material in the less compact state. Field observation reveals very few roots in the B horizon of these soils.

An apparent anomaly was observed by Veihmeyer and Hendrickson (70) in the seasonal change in moisture content of the subsoil of the Bale gravelly loam (California) under grapevines, and in that of a certain primary soil supporting a chaparral plant association. Despite water additions and plant transpiration these subsoils showed but slight changes with time in the content of total water, which suggested lack of absorption by roots. A density of 1.83 g. per cc. was observed in the Bale subsoil. Later work (*71*) was conducted in which the penetrability to sunflower roots was examined for several different manually compacted soils. The soils were compacted while moist to densities ranging from 1.46 to 2.06 g. per cc. At the beginning of the experiment they contained amounts of water calculated in all but one case to be in excess of their permanent wilting percentages, and in all cases to be well below saturation at the final, increased density sought; but no roots penetrated any of these soils and there was negligible loss of water from them. These authors have also observed little or no water extraction by pine trees, grapevines, fig trees, and chaparral shrubs from subsoils having the densities stated. No one density represented the limiting density for all soils examined, but results suggested that somewhat lower densities prevented root penetration more in fine than in coarse-textured soils.

SOIL AERATION

Soil aeration is the interchange of the soil atmosphere with the free atmosphere above the soil. Continued soil aeration is essential to remove the carbon dioxide produced by plants and microorganisms and to supply the oxygen needed by plant roots and the microorganisms in the soil. Obviously this interchange must occur in pores large enough that capillary forces do not keep them filled with water, and which have access to the atmosphere through a network of interconnected, unobstructed pore space channels. It has long been recognized that good soil aeration is needed for normal plant growth, and most farmers or gardeners are of the opinion that one of the chief purposes of tillage or drainage is to assure good soil aeration. In spite of this, comparatively little work has been done on the actual role of soil aeration in plant growth and little is known about the mechanics of the interchange of gases in the soil. Two facts have, however, been quite definitely established: oxygen is essential for normal root growth and extension, and oxygen is essential to the root if it is to carry out its normal function of absorbing nutrients and water.

Much of the work which has been done on the role of oxygen in root growth has been done in solution cultures. Admittedly, solution cultures and soil offer quite different environments for the growth of roots. Roots of different plants may develop and function differently in solution cultures than they do in soils. If, however, it is demonstrated that roots require oxygen for their normal functioning in solution culture, it seems legitimate to conclude that a similar requirement might be demonstrated in soil. Actually there is strong evidence for the essentiality of oxygen for root development in either type of environment.

Effect of soil aeration on root growth

Much of the solution culture work has been done with tomatoes. Arnon and Hoagland (*3*) obtained an average weight of 12.4 grams per plant for roots in unaerated culture solutions as compared with 19.9 grams per plant for aerated solutions. The yield of tomatoes for the aerated solution was 1.34 times that in the nonaerated solutions. Arrington and Shive (*4*) obtained from culture solutions dry-weight yields that were increased 210 per cent as the result of aeration. Erickson (*34*) too found that aerated plants were almost twice as large as those which were not aerated. Clark and Shive (*28*) and Stiles and Jorgensen (*66*) found that the dry weights of aerated roots were 1.6 times the weights of nonaerated roots, but Allison and Shive (*2*) found this ratio to be only 1.12 for soybeans. Most workers have pointed out that individual crops differ in their oxygen requirements, but none present clear-cut evidence that the roots of any plant can grow and function in the complete absence of oxygen.

Gilbert and Shive (*35*) give data for response to oxygen at various levels for different plants, and Erickson (*34*) gives an excellent recent review of the effects of oxygen in culture solutions. He found that root growth increased in proportion to the oxygen content of the culture medium.

Several observations of rooting habits in aerated versus nonaerated solutions are significant. Shive and his co-workers found that aerated roots were long, slender, and much branched. Erickson observed rooting habits similar to those reported by Bryant (*18*) who found that the length of aerated roots in his experiments averaged 37.4 cm., while those of nonaerated plants averaged only 10.9 cm. in length. The nonaerated roots were 15 per cent thicker than those aerated, and it was observed that aerated roots had a longer portion of the root tip over which absorption of water and salts could occur.

Similar results have been reported for plants grown in soils. Loehwing (*48*) found that soil aeration helped to produce plants having larger tops and roots, and that roots in aerated soils were distinctly more fibrous in character, longer, and more numerous than those from poorly aerated soils. He also found that roots grown in aerated soil had approximately twice the surface area of roots on plants grown in nonaerated soil (*47*). Knight (*44*) found that artificial aeration of soils increased top and root growth by 30 per cent in pot soil cultures. Baicourt and Allen (*5*) grew roses in aerated and nonaerated soils. They found that the aerated plants grew 68.4 inches, while the nonaerated plants grew only 37.3 inches in three months. Bushnell (*20*) aerated the soil in which potatoes were growing by placing a line of perforated tile in the soil. He obtained a yield increase of 15 to 29 per cent for aeration and observed that roots were more abundant around the tile line than in the main body of soil. A somewhat similar result was observed by one of the present authors for heavily watered tomatoes in a commercial greenhouse. Here growing roots were found only along the inside surface of the sterilizing tile, or spread out over the surface between the mulch and the soil. Presumably, deficient soil aeration caused by excessive watering prevented normal growth and development of roots in the main body of the soil.

Several experiments conducted in Ohio established a significant relationship between degree of soil aggregation, pore size, and crop yield, particularly on heavy clay soils. A high degree of soil aggregation or a fairly large proportion of larger pores was interpreted as indicating conditions favorable for good soil aeration. Baver and Farnsworth (*6*) found that, where total air capacity of the soil was only 3 per cent by volume, sugar beets suffered a 50 per cent loss in stand from black root rot disease and yielded only 2–4 tons. Very much higher yields of sugar beets were obtained after the introduction of cropping and management practices that increased the proportion of coarse pores present in the soil. Where air space porosity was raised, the loss in stand was reduced to only 10 per cent and the yield was raised to 12 tons. Page and Willard (*53*) found a fair degree of correlation between air space porosity and corn yield. Rotations that led to increased soil particle aggregation and, hence, better air space porosity and aeration were associated with large increases in corn yields, with average yields measuring from 49 bushels in 1936 to 84 bushels in 1945. They concluded that crop yields in their experiments were definitely limited by soil structure and aeration. Trogdon (*68*) found a highly significant correlation between yield of corn and air pore space in the soil, irrespective of the amounts or forms of fertilizer applied, provided essential elements were not lacking.

Cannon and his co-workers have conducted outstanding research on root–aeration relationships. Cannon and Free (*26*) stated: "Increased air supply to roots if not excessive favors root branching and probably accelerates the rate of root growth." They further suggested that aeration may be of equal importance ecologically with temperature and water supply. The effect of low oxygen levels and temperature on the growth rate of corn is brought out in Table I which gives some of Cannon's (*24*) results.

TABLE I

Effect of Oxygen Levels and Temperature on Rate of Growth of Corn (Cannon *24*)

Temp.	Rate of Growth of Corn With the Following Oxygen Levels in Soil Air 3 per cent	3.6 per cent	10 per cent
18° C.	1/3 normal	2/3 normal	normal
20°	1/5 normal		
30°	1/16 normal	1/3 normal	9/10 normal

It can be seen from Table I that there is a significant influence of temperature upon the relation of roots to oxygen. The effect of low oxygen levels is most pronounced at higher temperatures so that for best growth rates during hot weather, soil aeration must be excellent. Cannon (*22, 25*) also studied several species of plants differing in natural habitat and concluded: (*a*) complete absence of oxygen resulted in cessation of root growth; (*b*) many species can grow in an environment with as little as 0.5 per cent oxygen, but only very slowly; (*c*) rate of supply rather than partial pressure of oxygen governs growth rate; (*d*) when the supply of oxygen was too low to permit normal growth, the growth rate varied inversely with the temperature; (*e*) at constant temperature, growth varies directly with partial pressure of oxygen; and (*f*) there is a critical oxygen concentration for growth, but the extreme differences in critical concentrations of oxygen between species did not appear to be over 1 or 2 per cent.*

Another factor of considerable significance, reported by Cannon (*23, 21*), Bergman (*8*), Snow (*64*), Elliott (*33*), and Loehwing (*47, 48*), is that many plants have very few or no root hairs under conditions of low oxygen supply.

There is some evidence that when roots are deprived of oxygen or brought into an environment much lower in oxygen than normal, the roots may die, then be replaced by new, more stubby roots, or the roots may stop growing for a period and then start growing again very slowly. This would seem to indicate that when brought into conditions of poor aeration, some plants may undergo a change which adapts them better to partially anaerobic conditions, providing the demands on the roots are not too great during the period of adjustment. Much additional work needs to be done on this point to determine the behavior of plants when the soil on which they are growing is waterlogged either through heavy rains or improper irrigation. Went (75) reported results with tomatoes which suggest that if the plant can survive until a new root system can be established where the air supply is more favorable, the plant can continue to grow. He obtained increased growth with tomatoes in nonaerated nutrient solutions which had sent new roots into well-aerated moss (moistened with nutrient solution) tied around the stem above the level of the solution. There is considerable evidence that roots cannot function normally to absorb water and nutrients in the absence of oxygen. Thus, it does not appear that oxygen supplied to part of the root system of a plant would be sufficient to maintain the normal functions of the remaining nonaerated part of the root system. Instead, the old roots would die and the plant would become dependent upon the new root system. Boynton (*16*) found that few new roots were formed on apple trees when the oxygen level in the soil atmosphere fell below 15 per cent, and when oxygen was at 10 per cent or below and carbon dioxide was from 5–10 per cent, both

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*Cannon designated the lower critical pressure as that partial pressure at which growth ceases, and the upper critical pressure as that at which growth proceeds normally. Between these two values growth continues, but at a rate and in character which are subnormal.

root and top growth were seriously affected. Cannon (*24*) also reported that root growth (with corn) was below normal at oxygen concentrations of less than 10 per cent.

Since the carbon dioxide content of the soil atmosphere increases as the oxygen supply decreases, and carbon dioxide in rather high concentrations has an apparently toxic effect on roots, it has been suggested that the poor root growth associated with poor aeration is a result of carbon dioxide toxicity rather than lack of oxygen. Vlamis and Davis (*73*) found that when barley and tomato plants were exposed to carbon dioxide, a lethal effect was produced. Chang and Loomis (*27*) bubbled air, nitrogen, and carbon dioxide through culture solutions and found that carbon dioxide reduced both water and nutrient uptake. They also stated that toxic to slightly toxic concentrations of 10–20 per cent carbon dioxide are probably more commonplace in soils than limiting concentrations of 1–2 per cent oxygen. Parker (*55*), however, concluded that the carbon dioxide content of the soil is not important in influencing the absorption of inorganic nutrients by plants. Hoagland and Broyer (*39*) and Arrington and Shive (*4*) reached a similar conclusion for nutrient solutions. It thus appears that the main effect of carbon dioxide in most soils is that, when present in large amounts, oxygen tends to be deficient and, through pH changes, the solubility of certain nutrients may be adversely affected.

It seems amply apparent from the foregoing material that deficient oxygen in the soil would certainly limit the normal growth and extension of roots and development of root hairs and, hence, affect the availability of plant nutrients. Under specialized conditions, as in the greenhouses, otherwise normal plants are sometimes found growing with a very small root system where the soil is kept excessively wet and large quantities of fertilizers are used. The plants appear to be dependent upon oxygen dissolved in the irrigation water; this is in a sense a solution culture and in practice it is found that the plants are quite sensitive to any change in their environment. These plants can be grown with limited root systems where the soil functions chiefly as a support. It has not, however, been demonstrated that it is economical or desirable to grow plants in this way. For almost all soils, however, it appears that healthy, productive plants can only be expected when an extensive root system is established. Such root systems are only established in soils when soil aeration is good.

Loehwing (*48*) stated: "Improper composition of soil air manifests itself in reduced, slow-growing root systems, inadequate absorption, short-lived, discolored foliage and delay or failure of reproductive processes." Albert and Armstrong (*1*) found that fruit bud shedding and poor plant growth in cotton definitely resulted from poor soil aeration. Cannon (*24*) stated: ". . . it can be seen therefore, that there comes a point in the diminution of the oxygen content of the soil atmosphere when the growth of the root ceases because it is no longer sufficient to supply demands for energy correlated with physiological activities of higher temperatures." He stated further that ". . . in puddled soils with consequent poor aeration, and in summer, the matter of oxygen supply to the roots must be acute." He concluded that for corn to attain a fair rate of root growth at high temperature, aeration must be good indeed.

PHYSICAL PROPERTIES AND NUTRIENTS

The role played by soil clays in exchange reactions, and replenishment of the soil solution by replaceable metallic cations subsequent on their depletion by plants, is prima facie evidence of the significance of the soil particle-size distribution upon nutrient availability. This statement is not intended to minimize the fundamental importance of the mineralogical species present in the clay fraction, the possibility of nonreplaceable fixation of ions, or the degree of accessibility to solutions and living roots of the sites of exchange reactions. There is, however, much evidence of significant positive correlation between amounts of exchangeable cations and fineness of soil texture.

For illustration, reference may be made to analyses by Hosking (*43*) of the cation exchange capacity at pH 9 of a number of soils and their mechanical fractions from Australia and New Guinea. The soils represent a wide variety of genetic groups and lithologic origins. The exchange capacities per unit mass are by far the greatest for the material finer than 1 micron and, where observed in the coarser fractions, are, with apparent justification, attributed to incomplete dispersion. The exchange capacities of the whole soils, corrected for organic matter, increase with content of clay finer than 1 micron. This is particularly marked for those soils reported to contain some form of montmorillonite. In an earlier paper (*43*), Hosking expressed the opinion that unidentified amorphous inorganic materials with high exchange capacity may influence the exchange capacities of soils more often than is generally supposed.

Rubashov (*61*) working chiefly with certain beet-producing chernozems, of loess and "loesslike" origin, claims that the water-stable aggregates (7 mm. > 0.25 mm.) present possess physico-chemical properties distinctly different from the soils from which he isolated them. The amount of "humus" present in the surface 8 inches was found to increase with abundance of water-stable aggregates. It also tended to increase with the coarseness of the aggregates. There was some slight indication, further, that the amount of absorbed calcium may have been greater in the more highly aggregated soils. Aggregates coarser than 0.25 mm. (macroaggregates) separated from a given soil consistently contained more humus and absorbed calcium than those finer than 0.25 mm. (microaggregates) obtained from the same soil. This investigator (*60*) reported a similar relationship for podzolized soils. Improved structural quality, according to Rubashov, is associated with higher content of total humus and of "structure-forming humin substances."

The content of mobile nitrogen, acid-soluble phosphorus (Truog method), and exchangeable potassium in water-stable aggregates was found by Rubashov to be greater than in the soil as a whole. This was interpreted as indication that the water-stable aggregates strongly influence the nutritional value of a soil, as well as its physical and physico-chemical properties. Although more pronounced than those of the organic matter, the higher levels of these nutrients were associated with, and by Rubashov attributed to, the higher content and particular quality of the aggregate-forming organic matter.

Amongst many other experiments on the significance of different structural conditions in the soil, Doyarenko (see *45*) determined the amounts of nitrates found after six weeks in vessels of moist soil consisting of different aggregate size classes. Increases in nitrate formation paralleled increases in aggregate diameter and were attributed to the greater abundance of coarse ("noncapillary") pores amongst the coarse aggregates, which produced more favorable conditions for biological oxidation.

Rubashov's (*61*) results with respect to organic matter appear to be in general agreement with those obtained by Metzger and Hide (*50*) and by Weldon and Hide (*74*) who found, respectively, that organic carbon, and carbon and nitrogen content, were distinctly higher in well-aggregated than in poorly aggregated fractions separated from some Kansan soils. The percentages of carbon and nitrogen present, moreover, were found to decrease with a decrease in the size of aggregates except for the colloidal fraction, which was high in both elements. Applications of manure to alfalfa grown on one of the soils caused increases in the carbon content which were greater in the well-aggregated fraction.

These results differ from those of Clarke (*31*) who examined the aggregation and total nitrogen content of certain South Australian soils and concluded that nitrogen content is not significantly related to and has no significant effect on water-stable aggregation.

Breazeale and McGeorge (*17*) in one of a series of investigations on soil structure made by the soils group in Arizona, examined the effect of puddling upon microbial nitrogen transformations in some alkaline-calcareous soils. Puddling was brought about by soil manipulation, by means of an electrical vibrator, at the moisture equivalent. Nitrification was found to be completely stopped by such treatment during subsequent incubation, and denitrification became active. When nitrogen was originally added either as sodium nitrate or ammonium sulfate, a greater loss of nitrogen was observed from the puddled than from the unpuddled soil and the loss was entirely as gaseous nitrogen. Breazeale and McGeorge state that dry fallowing fully restores the nitrifying power of a puddled soil. Some benefit is obtained by the addition of a dust mulch to the surface of puddled soils. This is explained by upward movement, when the soil is moist, of dissolved ammonia into the layer of loose soil, and also by the somewhat more friable condition developed by the slower drying of the puddled soil itself. Both effects produce a condition more favorable to microbial nitrification.

In experiments upon the decomposition of organic matter in puddled and unpuddled Gila clay loam, McGeorge and Breazeale (*51*) found that addition of dry and rotted alfalfa to unpuddled soils resulted in excellent growth of barley seedlings. No nitrogen deficiency was apparent. Seedlings on the puddled soil made poor growth, were yellow, and appeared to lack both nitrogen and water. Puddling very seriously interfered with root growth. In one set of cultures, after incorporation of the alfalfa which underwent anaerobic decay in the subsequently puddled soil, the soil was allowed to dry thoroughly after planting; the growth was very poor. The unfavorable soil condition prevailed for a period of three months, as evidenced by unsatisfactory development of a second crop sown after that length of time. The results were attributed to the persistence of toxic products of anaerobic decomposition. These authors stated that "the productivity of puddled soils may be seriously reduced by the incorporation of organic matter while the puddled condition still exists" and, "in utilizing organic matter in the rebuilding of soil structure productivity may be lost, even though the structure is regained, if proper precautions are not taken in the use of organic matter."

Unsatisfactory response of tomato plants was obtained with treble superphosphate and both ammonia and nitrate nitrogen additions to puddled soils as compared with the same additions to the same soils in the unpuddled state. The effect of the puddled soil condition on nitrification (*17*) has already been mentioned. In the opinion of these investigators, lack of adequate aeration in the alkaline calcareous soils of Arizona may produce a deficiency in carbon dioxide with the resultant formation of normal carbonates and hydroxides. Plant growth is then affected by an unfavorable pH which produces a phosphate deficiency.

In other experiments McGeorge and Breazeale examined phosphate, potassium, and calcium uptake by means of Neubauer tests with rye. It was found that soil puddling reduced the availability of these nutrients.

Interpretation by Gorbunov of his experiments with nonreplaceable potassium (*36*) involves a relationship between soil structure and the availability of this element. Gorbunov estimated the amount of replaceable potassium in potassium-saturated samples of chernozem soils and a podzol clay by electrodialysis. Except for a control, the materials, after saturation and before electrodialysis, had been previously dried for 18 hours at different temperatures (40–105° C.) and, presumably, with different degrees of completeness thereby, before rewetting and maintaining wet for 72 hours. The electrodialysis which followed brought into solution, after about 2 to 3 hours and during the next 15 to 20 hour period, an amount of potassium which, in all cases, was greatest for the undried materials and which diminished as the temperature of drying increased.

In explanation, Gorbunov regarded the heating and drying as responsible for a process of aging of the colloidal material during which much of the diffuse part of the electrical double layer was destroyed. That is, combination of potassium ions with residual negative charges on the absorptive material made the latter less electronegative. He appears to have regarded this change as in large part irreversible and accompanied by the formation of microaggregates with reduced dispersibility. According to this hypothesis, aggregation may hinder the replaceability of the ion concerned, in this case potassium. Gorbunov made no statements concerning the size of the "microaggregates." On the other hand, he considered that organic matter plays an important role in the fixation process. Gorbunov's hypothesis does not satisfactorily explain the relationships reported by Rubashov (*61*) between aggregate size and replaceable potassium. Martin, Overstreet, and Hoagland (*49*), on the other hand, found that potassium fixation may be considerable in moist soils and, contrary to Gorbunov, do not consider that drying is directly involved. The clarification of these apparent inconsistencies awaits more work by chemists and physicists on the mechanisms of the aggregation process.

Experiments were recently conducted by Greacen (*37*) upon the applicability of Boyd's equations (*15*) to cation exchange in columns of soil aggregates. Greacen gave special attention to mechanisms which might control rates of exchange between soil-adsorbed cations and cations present in dilute, permeating solutions. Rates at which soil aggregates of different sizes released their cations were measured by passing solutions at controlled velocities through very short columns of aggregates.

Cation exchange for the Yolo and Aiken soil aggregates within the "fine" size class, 0.3–0.1 mm., was found to be an equilibrium process during passage, through a column of calcium aggregates, of $N/50$ magnesium chloride solutions at macroscopic velocities of less than 0.3 cm. per second. For coarser aggregates, > 0.6 mm., of Aiken soil, diffusion of ions through the individual aggregates appeared to control the rate of exchange, for macroscopic velocities in excess of 0.004 cm. per second. The two soils did not behave identically and, for soils in general, much individuality of behavior seems probable.

Rates of internal diffusion were found to limit the exchange rates with the fine aggregates also, provided that the macroscopic velocity of the permeating fluid was made sufficiently great (e.g., 1–2 cm./sec.). Such high velocity, however, is unlikely to be of direct interest in problems of plant growth in soils.

From these experiments it appears that ionic diffusion rates within aggregates may possibly affect the ready availability of exchangeable cations, particularly if the aggregates are large, impenetrable by roots, and not easily dispersible.

ROLE OF SOIL AERATION IN NUTRIENT UPTAKE

Soil aeration not only affects the extent and character of root growth, but is also of extreme importance through the effect oxygen has upon the assimilation of nutrients by roots. Much of the more conclusive evidence again is to be found from studies in solution cultures, primarily with excised root systems. Since this work will be the topic for another paper on this symposium, it will not be reviewed in detail here, but a few papers will be cited to indicate the essentiality of oxygen in absorption. The work of Steward and his colleagues (*65*) showed that, for tissues, aerobic respiration supplies the energy necessary for salt absorption against a concentration gradient. The extensive studies of Hoagland and Broyer (*39, 40*) proved that salt accumulation by roots is also dependent upon aerobic metabolism, and oxygen is one of the indispensable requirements for salt accumulation (movement of salt against a gradient) by excised barley roots. These studies were made on assimilation of potassium, halide, and nitrate. They also showed that a relatively high concentration of carbon dioxide is required to greatly depress salt accumulation. Where oxygen had been carefully excluded, they did not observe accumulation of salt against a gradient. The recent work on this problem is excellently summarized by Hoagland (*41*).

In work with growing tomato plants in solution cultures, Arnon and Hoagland (*3*) found that roughly 1.4 times as much each of potassium, nitrate, phosphate, calcium, and magnesium was taken up from aerated solutions as from nonaerated solutions. The size of plants and yield of fruits were correspondingly high. Arrington and Shive (*4*) found that absorption of ammonia nitrogen was 1.34, and of nitrate nitrogen 1.30, times greater from aerated than from nonaerated solutions. Pepkowitz and Shive (*56*) found that absorption of calcium and phosphorus was directly dependent upon dissolved oxygen supply, whereas absorption of potassium was not so materially influenced. Chang and Loomis (*27*), on the other hand, found that aeration increased absorption of potassium most, followed, in order, by nitrogen, phosphorus, calcium, and magnesium.

Direct evidence for the effect of soil aeration on nutrient availability is scarce, but the evidence available is significant and highly suggestive. Probably the main reason why there are few data on this subject is that there is no good way of measuring or characterizing soil aeration; thus, it has been difficult to prove that certain conditions observed in the field have been caused by poor soil aeration. Many attempts have been made to measure the partial pressure of oxygen in the soil atmosphere directly, but little success has been attained. One difficulty has been that the analytical methods available required a rather large gas sample. This was not easy to extract from the soil in a condition representative of the atmosphere surrounding plant roots. Another difficulty is that measurement of oxygen in the soil air may not indicate the amount of oxygen dissolved in the water bathing individual roots, for normal soil changes in the oxygen concentration of the soil atmosphere may occur too rapidly for equilibrium between dissolved and gaseous oxygen to be maintained. With the recent introduction of the Pauling oxygen analyzer, which requires only a few cubic centimeters of gas and which indicates partial pressure of oxygen directly and automatically, a new tool is available which should give valuable information concerning the mechanism of gas interchange in the soil.

Even without data on the actual aeration status of the soil, a few experiments and observations have been reported which emphasize the importance of aeration to normal plant growth and the effect of inadequate soil aeration upon nutrient availability. Page and Willard (*54*) observed in an experiment which compared different tillage methods for corn, that marked potassium deficiency symptoms occurred in corn on plots which had been prepared by disking only. Milder deficiency symptoms were observed on these same plots even where 300 pounds of 0–14–7 had been placed in the row at planting time. The air space porosity in these plots was only 14.2 per cent as compared with 26.9 per cent for adjacent plowed plots, which showed no potassium deficiency symptoms. These two adjacent plots were comparable except for the one variable of tillage in preparing seedbeds.

Bower, Browning, and Norton (*14*) obtained symptoms of both nitrogen and potassium deficiencies in the first reported experiment which demonstrated that tillage had a direct effect upon availability of nutrients. They observed definite nitrogen deficiency symptoms on corn which was growing on land prepared by disking or subsurface tillage, even where nitrogen fertilizer was applied. Corn on plowed land showed almost no evidence of nitrogen deficiency. Similar results were obtained with potassium, but the differences were not quite so clear cut. Where no fertilizer was applied, however, the potassium content of plowed corn was 70 per cent higher than the average of corn grown by the other three treatments. Significantly, they found no difference in exchangeable potassium content of the soils between these plots, yet the potassium was definitely more available to the corn plant from the plowed plots. They indicated that tests showed more ferrous iron (indicating lack of aeration or reducing conditions) in the soils of those plots which were not plowed and on which potassium deficiency was observed.

Lawton (*46*) made a study of the effect of aeration on absorption of nutrients in pots in the greenhouse, where different degrees of compaction, different water levels, or forced aeration could be maintained. He found that where pore space was reduced, either by compaction or increase in water content, absorption of potassium was much less than from a normal soil. Under these conditions root growth was also seriously reduced. Forced aeration eliminated the distinct potassium deficiencies, even though air was forced through the soil for only 30 minutes a day. He observed that absorption of potassium is more dependent upon soil aeration than is uptake of nitrogen, calcium, magnesium, or phosphorus. This is in agreement with the findings of several investigators working with solution cultures.

Smith and Cook (*63*) studied the effect of soil aeration and compaction on nitrification and the growth of sugar beets. They found that in every case compaction decreased beet yields, and artificial aeration tended to overcome the harmful effects of compaction, indicating that low aeration was limiting yields. They also found that compacted soil, in which large amounts of nitrogenous material had been incorporated, had been prevented from rendering these nitrogenous substances available as nitrates, at least to a level comparable with that which might normally be expected and which was demonstrated on the same soil that had not been compacted.

Trogdon (*68*) found in a study of effectiveness of various nitrogen fertilizers that maximum utilization of added fertilizer elements was not obtained if air space porosity was low. Wherever soil porosities were low, tests showed the presence of reducing conditions in the soil throughout much of the two rather wet seasons and definite phosphorus and nitrate deficiency symptoms were observed even where 700 pounds of ammonium sulfate and 800 pounds of 0–10–10 fertilizer had been applied.

It is, of course, well known that oxygen is required for the production of nitrates, for the fixation of nitrogen, and, in fact, for the continued activity of most of the important types of microorganisms in the soil. A fact which is not so widely considered, however, is that the requirement for oxygen by microorganisms which are actively decomposing organic matter in the soil is comparatively high. It appears very likely, in fact, that the commonly observed depression in plant growth immediately after adding large amounts of green manure or other organic matter to a soil may be due more to competition between the higher plants and the microorganisms for the limited supply of oxygen than to any other single factor. The full significance of this point remains to be worked out, but it is worthy of further study. Trogdon (*68*) found that, where large quantities of fresh organic matter were added to soil in contact with fertilizer banded below the surface, there was a reduction in yield as compared to no organic matter. This was apparently associated with deficient oxygen supplies since definite nitrogen, phosphorus, and potassium deficiency symptoms were observed in the plants and reducing conditions could be demonstrated in the soil near the organic matter. It is likely that some of the peculiar results sometimes observed in glass-house vegetable production can be explained as due to temporary partial reducing conditions brought about by turning under large amounts of organic matter where temperature and moisture conditions favor rapid decomposition.

Where reducing conditions are developed in the soil either through additions of large amounts of organic matter, through waterlogging, or compaction, or a combination of these factors, it is, of course, well known that important chemical changes are produced in the system. Under reducing conditions, carbon dioxide is usually reduced to methane; nitrates to nitrites, ammonia, or free nitrogen; sulfates to hydrogen sulfide; ferric to ferrous iron; and trivalent to divalent manganese. There is considerable evidence that most of the reduction is brought about by microorganisms and that the presence of readily decomposable organic matter greatly favors the development of reducing conditions wherever oxygen supply might be low. In most soils, however, every effort is, or should be, made to prevent the occurrence of extreme reducing conditions, so that too great emphasis on the nature of the chemical status of the reduced soil is probably not warranted for this discussion.

Of more significance is the effect of even a partial reduction in the supply of oxygen available to plant roots, caused either through utilization of available oxygen by microorganisms, or through reduction in the rate of supply through the soil pores. Since oxygen is necessary for normal root functioning and absorption of water and nutrients, any process which significantly reduces the supply will have an adverse effect on root growth and availability of nutrient elements.

MECHANICS OF SOIL AERATION

The work reported in the preceding sections indicates that nutrient uptake and availability, particularly of potassium, is affected by soil aeration. As was mentioned earlier, the detailed mechanics of soil aeration are not well understood, but workers in the field are quite well agreed that practically all aeration occurring in the soil results from gaseous interchange by diffusion. Oxygen diffuses into the soil and carbon dioxide diffuses out, simultaneously. Factors such as wind, barometric pressure changes, or flushing by light rains or irrigations, apparently contribute little to the movement of soil gases. In the past, attempts at studying soil aeration have been directed mostly toward studying partial pressures of oxygen in the soil air, by measuring porosity (usually at a standard moisture content) or by studying permeability of soil to gases under a pressure differential. Each of these methods has distinct shortcomings. Difficulties in measuring oxygen have already been mentioned, but such work should be continued making use of the newly-available oxygen analyzers. Determination of porosity gives at best an indirect measure of aeration of a soil, particularly if all measurements are made at some standard moisture condition, since this exact moisture condition may only occur in the field rarely and, hence, cannot indicate the aeration conditions under which the plants are living. An improvement in this measurement is made possible by measuring porosity at field moisture with the pressure pycnometer (52). With this instrument it is possible to measure the pore space actually filled with air at the moisture condition prevailing in the soil at the time of sampling. Another shortcoming of porosity measurements is that the effectiveness of air pore space in the soil is limited by the degree to which there are open channels or continuous pores to and through the surface so that free diffusion can occur. It is easy to see how a soil with apparently very favorable porosity in the root zone could be quite unsatisfactory for root growth if the surface were sealed through formation of a crust. Possibly the most important benefit of a mulch comes through its action in maintaining porosity unblocked or sealed through to the surface, so that free diffusion into the soil can occur. The chief drawback to characterizing aeration in terms of air permeability is that gas flowing under a pressure differential may be subject to such factors as turbulent flow, friction, and others which are not of significance in free diffusion.

In recent work, Blake (*9, 10*) has studied the diffusion process. He found, as have previous workers, that there is a linear relationship between diffusion and porosity, but, unlike most work reported in the literature, he did not find a constant factor to express the relationship between porosity and diffusion. The coefficient was different for soils of different properties. Thus, two soils might have identical porosities when expressed on a volume basis, but, if they differ in the character of the pores or in the arrangement of the pore spaces, they might behave quite differently in so far as diffusion, and hence aeration, is concerned. One soil having 30 per cent air space porosity with interconnected channels open to the atmosphere would certainly be better aerated than another having the same percentage porosity but having few interconnected channels. It was suggested, in fact, that measuring the diffusion characteristics of a soil and evaluating porosity in terms of the diffusion process rather than on a strict volume percentage basis offers a most promising method of attack on the difficult problems in soil aeration.

To accomplish this and make an even more direct determination, a new method of measurement was devised. This procedure has not yet been described in the literature, but it appears to have considerable promise and value as a research tool. Briefly, it consists of introducing a large excess of carbon dioxide into a soil from a cylinder of gas (the gas is introduced through a metal capillary tube arranged into a pointed sampler) to sweep out soil gases from a rather large volume of the soil. The amount of gas is not critical as long as an excess is used. When the flow of gas has been stopped, the oxygen analyzer is attached to the same tube and small samples are withdrawn as frequently as desired. By this means it is possible to measure the rate at which oxygen diffuses into the soil to dilute the carbon dioxide until normal soil atmosphere concentration is approached. The results are expressed as a time curve showing renewal rate for diffusion of oxygen into the soil. The method is simple and direct: no foreign materials are introduced, diffusion proceeds unhampered through natural soil pores at existing field moisture content, the results are easily interpreted, and the sampling site is not changed, so that measurements can be repeated at the same location as often as desired throughout a whole growing season. A similar procedure which uses standard 3-inch cores of soil in natural structure has also been used in the laboratory.

Even with a limited number of determinations, some interesting data were obtained with the techniques described. Thus, renewal rate—the time for oxygen to reach the normal concentration usually found in the soil—was two hours in a freshly plowed soil and three hours in a corn plot (determinations made in late fall); at four hours the oxygen level had reached only 16 per cent (instead of the usual 18.5–19.5 per cent) in an alfalfa–brome-grass plot, and in continuous bluegrass plot the oxygen level had risen only to 14 per cent in 22 hours. Temperatures were low, and it was not thought that production of carbon dioxide in the soil was a significant factor, although it is a remote possibility in these experiments. It is surprising to note that renewal rates are considerably lower than published estimates have suggested.

Direct determinations of oxygen in the soil atmosphere with the oxygen meter have shown very few oxygen concentrations lower than 18.5 per cent. Even on experimental plots (52, 53) where soil structure was known to be poor and where crop yields were definitely limited by poor aeration, measured oxygen levels were comparatively high. It was concluded that on most soils aeration is adequate during much of the season when the soil water is at or below field capacity. At times of rains or heavy irrigations soils of good structure drain readily, and normal aeration is re-established before the supply of dissolved oxygen in the soil water is exhausted by the plant. In soils of poor structure, however, aeration may not be so rapidly re-established, and, if the oxygen supply runs out and is deficient for any length of time, the plant may undergo a shock or change which will adversely affect its future growth and development. Much work needs to be done on the response of plants to intermittent periods of low oxygen supply, to determine the extent to which root growth and absorption of nutrients are affected.

As an example of a situation in which plant growth is definitely limited by physical properties, and to show that these properties are subject to modification, reference will be made to the soil structure rotations of the Ohio Station (52, 53). Various rotations were established in 1936 on Paulding clay to compare the effects of different cropping systems on soil structure. Summarized results are presented in Table II. It can be seen that "structure building" rotations which include sod crops have effected an improvement in productive capacity of the soil while, with those rotations which do not provide for adequate organic matter return, productivity has sharply declined.

The changes described have been brought about in a comparatively short time. The effect of fertilizers has been studied on the same plots but no consistent or large increases have been obtained; in fact, in some years there was a loss in yield where fertilizers were added. Table III shows the average corn yields and gains from fertilizers in recent years. It is significant to note that the natural fertility status of these soils is high: yields as high as 110 bushels of corn have been obtained when the season and the physical condition of the soil were both favorable.

TABLE II

Relative Average Unfertilized Corn Yields for Different Rotations
(Paulding, Ohio)
(Actual 1936 Yield = 100 per cent)

Rotations	1945	1942–1948
	per cent	per cent
4 year (corn, oats, 2 yr. alfalfa or alfalfa-brome grass)	191	147
3 year (corn, oats, alfalfa or sweet clover)	147	106
2 year (corn, oats)	90	71
Continuous corn	61	36

Note: 1945 best corn year during period. Data before 1942 not used because rotations had not been well established before that year (53).

TABLE III

Average Unfertilized Corn Yields and Response to Fertilizer Applications
(Paulding, Ohio)

Rotations	Av. Yield 1945, 1946, 1948	Gain for Fertilizer
	bu. per acre	bu. per acre
4 yr. (corn, oats, 2 yr. alfalfa or alfalfa-brome grass)	62.5	3.9
3 yr. (corn, oats, alfalfa or sweet clover)	56.1	1.4
2 yr. (corn, oats)	36.6	8.7
Continuous corn	16.3	8.2

Note: 1947 yields not included since yield data were not taken for fertilized vs. nonfertilized plots.
Fertilizer schedule: 150 lb. 0-20-20/acre/yr. plus 300 lb. 20-0-0 before corn on last two rotations.

FIGURE 1. Appearance of structure rotation plots on July 20, 1949, Paulding, Ohio. *Upper:* corn, oats, two-year alfalfa rotation. *Lower:* continuous corn. Poor drainage characteristics and poor soil structure, which have developed since 1936, definitely limit plant growth through poor soil aeration on this plot; there has been a definite improvement in soil structure and, hence, in soil productivity on the plot shown at the top.

These yields were obtained in areas which had received no fertilizers for several years. Yields as low as 5 bushels have been obtained from plots where soil structure has been allowed to deteriorate. Fertilizer application at the rate shown in Table III raised the yield on the corresponding plot in that year to only 11 bushels. The soils under different rotations show very great differences in physical properties, especially as regards drainage. The conditions of soil and crops shown in Figure 1 and the measured physical properties including porosity, aggregation, and diffusion all correlate well with yields, indicating that the yields are definitely limited by poor soil aeration. On the continuous corn plots the corn is very poor and shows many deficiency symptoms for the various nutrients even though these plots regularly receive 150 pounds per acre of 0–20–20 and 300 pounds of 20–0–0. In this case physical properties of a soil definitely limit the growth of the plants and the over-all availability of the plant nutrients, and addition of fertilizers does not raise the yield to a level where farming would be profitable. These studies furnish a striking example of the inadequacy of a strictly chemical approach in studies on crop production and emphasize the close interrelation between all plant growth factors, physical as well as chemical.

To summarize, oxygen or proper soil aeration is definitely needed for normal root growth, and for normal absorption of nutrients by roots. Nutrients may be abundant and available as shown by chemical tests, but largely unavailable to plants if the soil air supply is not adequate. Soil structure is the governing property which not only controls air supply but also affects the growth of roots as they penetrate the soil. Large amounts of organic matter, added for the purpose of improving soil structure, may cause temporarily harmful rather than beneficial effects through production of reducing conditions in the soil. Thus, soil structure must be taken into account in interpreting the results of fertilizer trials or applications, and at the same time it must be realized that soils can be improved not alone through addition of fertilizers but through improvement of their physical properties as well.

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CHAPTER

7 Role of Soil Microorganisms in Nutrient Availability

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SOIL MICROBIOLOGY is a field of endeavor about which considerable diversity of opinion has been expressed by workers whose interests lie in the study of factors involved in plant growth. Many completely ignore the soil microbial population, apparently because it has been established by nutrient culture studies that, under certain peculiar circumstances not normally achieved in soil, the presence of microorganisms is not essential for optimum plant growth. Others refer to the soil population only when unsatisfactory plant growth is obtained; it is then convenient to be able to ascribe the growth deficiency to "immobilization" of this or that essential nutrient by the soil microorganisms.

To a degree the views of some of the earlier soil microbiologists may have contributed to this attitude. Particular groups of microorganisms, studied in pure culture, were found to bring about reactions the product or products of which in soil might advantageously affect plant growth. These were "beneficial" organisms. Others, capable of effecting transformations that might result in reduction in solubility, availability, or loss of some nutrient, were "harmful" organisms. The great bulk of the population not having obvious specialist functions was largely passed over. It was implied that in some way or other the role of the soil population with respect to plant growth could be expressed by a summation of the positive (beneficial) and negative (harmful) components.

In this review consideration will be given only to the activities of microorganisms that affect the supply of major and minor plans nutrients in soils. Direct effects other than nutritional, or indirect effects such as those that relate to soil aggregation, will not be discussed.

THE NATURE OF THE SOIL POPULATION

As will be developed later, the vegetation supported by the soil and the microbial population within the soil each exert influences on the other. The relationship is peculiarly complex and cannot be readily described or defined in a single term. The activities and requirements of the plants mesh with the activities and requirements of the microorganisms at many points. At some points these two populations may be competitive; at others the activities of the one may help to satisfy some requirement of the other; elsewhere they may be compatible or inert.

As Truog has pointed out, the "living phase" of the soil, its microbial population, is diverse in character. Many of the specialist organisms in soil, some autotrophs and some heterotrophs, were discovered fairly early in the development of soil microbiology. Because almost all of these were bacteria, the impression has developed that in so far as the growth of plants is concerned, the bacterial flora of soil is the significant flora and that other groups of microorganisms, being more or less inert in their relationships to plant growth, can be safely ignored. Soil microbiology, however, is certainly not a subfield of bacteriology. Plating experiments and pseudoquantitative ecological studies on the soil population undoubtedly show that the bacteria are numerically dominant. They are, however, individually small in size and may not, therefore, occupy so dominant a position with respect to the total mass of the population as their profusion might suggest. This may particularly be the case in soils or horizons of soils in which there have been recent additions of residues of crops or other vegetation. Filamentous forms—fungi and actinomycetes—may then in mass constitute a major part of the active population. The available quantitative information about such forms is of dubious value. Plate counts of organisms with an indeterminate habit of growth are largely meaningless because they represent as individuals both spores and hyphal fragments. In certain soils, and particularly those of lacustrine origin, algae may not infrequently be quite numerous. Protozoan forms can often be seen by direct microscopic examination of soil and a large number of genera of protozoa have been isolated from soil. The role of these two groups in the composite activity of the complex microbial population of which they are a part is completely obscure, and, since the abandonment of the "protozoan" theory of soil fertility and the censuslike studies almost twenty-five years ago, both have been largely ignored.

A soil is a product of its environment and the populations which develop within the various horizons of a soil are differentiating characteristics no less valid than those exhibited by the organic or inorganic components of the soil. They are, however, far less readily determined or simply described and, moreover, they are dynamic and in a sense easily mutable. Particularly is this the case in cropped soils. In virgin soils or under permanent sod there is no doubt a greater degree of population stability.

Soil is a medium in which many types of organisms appear to be able to retain viability for long periods though inactive. This is as true of vegetative cells as it is of bacterial endospores or the spores of fungi or actinomycetes. Perhaps the retention of water by clay surfaces provides a shell that is protective to organisms in contact with the clay. There is no ready way of determining whether an organism isolated from soil was active immediately prior to isolation or was in an inactive, dormant condition. The picture of any soil population that can be obtained is a highly indiscriminate one, and accordingly it is not easy to ascertain the ecological responses to cropping or even to major changes in the physical, chemical, or nutritional status of a soil.

Even uncropped or untilled soil does not provide a biologically stable environment. Even though the carbonaceous energy supply in such soil is only occasionally supplemented, the physical environment is far from constant. There are seasonal and diurnal temperature changes, and, perhaps more importantly, there are the continually occurring erratic fluctuations in moisture content imposed by rainfall and evaporation. In the higher moisture range, particularly in fine textured soils, gas diffusion may be impeded, which results in alteration of the composition of the soil atmosphere and, in the extreme case of waterlogging, in the establishment of reducing conditions and the dominance of facultative and anaerobic bacteria.

THE NUTRITIONAL REQUIREMENTS OF SOIL ORGANISMS

In examining the question as to the effects which soil organisms may have on the availability and supply of plant nutrients, some consideration must first be given to the comparative nutritional requirements of microorganisms and plants in order to determine whether they overlap and, if so, to what extent and in what circumstances they may be competitive. Plants in general and crop plants in particular, despite all their diversities of form and structure, probably have a greater similarity of requirements than do the heterogenous forms of life that constitute the microbial population of the soil. Plants and microorganisms probably require the same major nutrient elements, though no doubt wide quantitative differences occur in the levels of poverty adjustment and luxury consumption. The qualitative and quantitative range of essential minor elements is less well established, particularly for microorganisms. Plants, in the language of the microbiologist, are photoautotrophic organisms; the soil population on the other hand is predominantly heterotrophic, though there are some important groups of chemoautotrophs, and some photoautotrophs that probably live heterotrophically. The plant can and normally does satisfy its nutrient requirements from inorganic sources. Many heterotrophic organisms also can satisfy their nutrient requirements exclusively from inorganic sources provided that an organic source of carbon and energy is available, so, in this respect, these organisms may be said to be capable of competing with plants for the available supply of mineral nutrients. Many heterotrophic organisms, however, are able to utilize organic sources of nitrogen, phosphorus, sulfur, and, no doubt, other elements. In general these are not utilized directly but only after hydrolysis or other degradative transformation that may result in the liberation of the nutrient element in an inorganic form. Some soil organisms require an organic nitrogen source, usually some simple amino acid, and, indeed, attempts have been made to use nitrogen nutrition as an aid in characterizing different soil populations (*16*). There is evidence that certain legumes under sterile conditions can directly utilize some soluble organic sources of nitrogen, such as aspartic acid (*15*); apparent utilization of organic nitrogen sources by nonlegumes has also been reported, but the maintenance of a condition of asepsis that would rule out the intervention of organisms on the surfaces of the roots has not been indubitably established. There are reserves of phosphorus and sulfur in soils in forms other than organic, so the fact that organic sources of these elements may be available to microorganisms but not to higher plants is not of special significance. There is, however, no reserve of nitrogen in soils other than that in the organic form. It might, therefore, be supposed that the soil population could successfully compete with the plant for nitrogen and that the supply to the plant would be limited thereby. This is rarely the case, however, as will be discussed later.

In so far as the mechanism of uptake of nutrient elements is concerned, it would seem that the process in plants and microorganisms is essentially similar. The clay fraction of soil is now recognized as being of great significance in plant growth. The physical and nutritional characteristics of a soil are determined to a large degree by the physicochemical properties of the clay components. The organic fractions, though constituting a vital and dynamic reserve of certain nutrients, modify but cannot fundamentally alter the character of a soil imposed on it by the presence of particular clay minerals. Microorganisms in close contact with the clay colloids can no doubt compete with the clay for cations in the soil solution and by contact exchange can accept ions adsorbed on the clay. Certain soil organisms have indeed been shown to make better growth in a clay-containing medium than in conventional mineral nutrient media (8). Although this may not be due exclusively to the form of presentation and means of transfer of ions, nevertheless it appears probable that in soil, microorganisms absorb ions by a process similar to that which holds for plant roots, and in this respect they can be considered to be competitive for exchangeable ions. The cation adsorptive capacity of some bacteria has been shown experimentally to be several times that of an equal mass of barley roots (7). This observation may not be of particular significance because not all retentive positions have to be occupied by cations for growth to occur. Viability may be retained even though all cations are replaced by hydrogen or methylene blue. It appears likely, then, that bacteria and plant roots in the same absorption zone are in equilibrium with each other and with inorganic or organic colloids with respect to cation distribution.

EFFECTS OF THE CROP ON THE SOIL POPULATION

Although the main topic of this paper is the relationship between soil microorganisms and plant nutrition, it is pertinent to inquire just what effects may be impressed upon the microbial population of the soil by the presence of a crop. In other words, this amounts to an inquiry as to whether the population of an uncropped soil would be modified in any way by the presence of a crop. All techniques of study show that in the immediate vicinity of the root hairs, rootlets, and roots there is a great concentration of organisms, primarily bacteria, and that the root system of plants is, therefore, virtually encompassed by a zone in which microbial activities are presumably intense and are not necessarily identical with those occurring in soil more remote from the roots (*2, 4*). The biochemical activities of the rhizosphere population are somewhat obscure and yet highly pertinent to the general topic under consideration. It seems to be established that the population of the rhizosphere is not merely a more concentrated and more active version of that to be found in the soil away from the roots. Qualitative as well as quantitative differences are found. Bacteria greatly predominate over fungi or actinomycetes; gram negative rods constitute the bulk of the population; sporeformers are few. The high concentration of organisms in this zone is particularly striking. Although there is great variation in figures reported by different workers, according to particular circumstances, the rhizosphere contains 5–50 times as many bacteria countable on plates as are found outside this zone. The question might therefore be asked as to whether there is much point to the study of the general soil population; perhaps the rhizosphere population is the only significant one in so far as the growth of the plant is concerned. Such an answer would ignore the fact that the root zone constitutes only a fraction of the soil under any crop other than a permanent sod. It would ignore those biochemical activities that precede the establishment of a particular crop on the land and its invasion by roots. It would ignore the fact that no new organisms are ordinarily introduced when a crop is planted. It would ignore the fact that when a crop is harvested the rhizosphere population does not immediately disappear; activities in the vicinity of the roots remain intense though now root decomposition occurs.

Any concentration of microorganisms in soil is an indication of the presence of available energy material. The high concentration of organisms in the vicinity of roots is not easily explained unless normal plant roots in soil lose soluble organic substances. There are, of course, abraded root caps and sloughed-off epidermal cells, but these seem inadequate to account for the distribution of rhizosphere organisms encountered around roots of all ages and all species, and for the clear qualitative differences that are detectable among the rhizosphere populations of different species. Root excretions of various sorts have been detected in a number of special cases. Toxic secretions have been said to be responsible for "soil fatigue," and for the effect of one crop on another succeeding it, though microbiological and nutritional factors have not always been excluded (*14*). Root excretions, either toxic or nontoxic, would be likely to be detected in soil only if they were unavailable or only slowly available as energy sources for microorganisms. It is now well established that certain inorganic ions may be lost from plants, particularly as maturity is approached, but the technical difficulties of studying under aseptic conditions the excretion of organic substances seem to have discouraged investigation. Claims have been made, and some evidence presented, that sugar and carboxylic acids, such as malic acid, may pass out of roots of certain plants, and amino acids out of legumes (*5, 8*). Certain enzymes, presumably of plant origin, also have been identified in the vicinity of roots. On microbiological grounds there would certainly appear to be support, admittedly inferential, for the view that some energy source, other than cellular debris, originates in the roots of the growing plant and causes the development of the microbial mantle which ensheathes the roots of plants in soil.

EFFECTS OF MICROBIAL ACTIVITY ON SOLUBILITY OF NUTRIENT ELEMENTS

Microorganisms may affect the nutrient supply to higher plants in a number of ways. Most reviews and textbooks put emphasis on indirect and inadvertent effects on the solubility of many elements. In addition, it is pointed out, quite correctly, that there are a limited number of specific inorganic transformations of nutritional importance carried out by autotrophic organisms. Elements such as nitrogen, sulfur, and iron in various forms are involved. These are ordinarily relatively easy to determine; the significance of the transformation and its quantitative aspects can be evaluated. Those indirect effects on solubility of other elements said to be due to products of metabolism of microorganisms are much more difficult to assess. The clay components of the soil and the less weathered rock fragments contain many elements required or utilized by plants in varying amounts. Some of these may occupy positions in the lattice of the clay or silicate; others may be cations retained by the clay or organic matter. At various times investigations have shown that the maintenance of carbon dioxide concentrations in soil may result in an increase in the availability of certain elements. Some of these experiments have been quite artificial, in that lengthy extractions with carbon dioxide–saturated water have been carried out. In others, however, gaseous carbon dioxide has been passed through the soil and changes in availability have been measured by plant uptake. More attention has perhaps been given to phosphate in such studies than to other elements, and it appears to be established that increased solubility of phosphate from rock phosphate or basic calcium phosphate may result, especially in neutral or calcareous soils. The amount of carbon dioxide evolved in soil by microbiological activity is substantial. Under optimum conditions in soils well supplied with organic matter, carbon dioxide evolution may attain rates as high as 100 pounds per acre per day, though figures of 20–30 pounds per acre per day are more general. Any increase in the hydrogen ion concentration might be expected to increase the solubility of bases such as potassium and magnesium, but, as Dr. Jenny indicates, it is not now assumed that these must be in the soil solution to be available to the plant. Moreover, experiments with plants growing in electrodialyzed colloidal clay have shown a greater availability of certain elements, such as iron, aluminium, and silicon as measured by plant uptake, than pass into solution as a result of long carbon dioxide saturation of the clay suspension.

Much is sometimes made of the presence of organic acids in soils. Organic acids, however, are not a usual product of bacterial activity under aerobic conditions. They may be produced by certain fungi, but as they themselves are readily available sources of energy other organisms would immediately accomplish their oxidation. Under anaerobic conditions, on the other hand, soluble organic acids may accumulate since they form characteristic products of the activity of facultative and anaerobic forms. Polysaccharides containing carboxyl groups, such as the polyuronide gums produced by some bacteria which utilize cellulose, may represent the only "acids" that accumulate under aerobic conditions (*10*).

Continually cropped soils in humid regions become increasingly more acid. The change in base status in such soils is due not only to cation removal in the crop and to the formation of carbonates or bicarbonates; the nitrification process, which is believed to be exclusively microbiological, is in part responsible. Ammonia, liberated from organic sources, which at first probably occupies positions on the base exchange complex, may be oxidized to nitric acid and then combine with other bases. The oxidation of 100 pounds of nitrogen, an amount often exceeded per acre per season, would result in the formation of 450 pounds of nitric acid. Similarly, the oxidation of the sulfur present in proteins and thio compounds results in the formation of sulfuric acid.

It is unlikely, however, that the direct and indirect effects of microbial activity on nutrient availability that result from the production of carbon dioxide, carboxyl groups, or inorganic acids rival in importance those transformations that are involved in the decomposition of organic matter and plant residues.

ORGANIC MATTER DECOMPOSITION AND MICROBIAL SYNTHESIS

The soil population is predominantly heterotrophic and accordingly the supply of energy is of major significance in determining its over-all activity. Soil organisms constitute an important link in the chain of carbon transformations in nature. The green plant accomplishes the synthesis of a great diversity of organic substances from carbon dioxide and water; the soil organisms carry out the reverse process and oxidize to carbon dioxide and water almost any carbonaceous material that finds its way onto or into the soil. That these downgrade processes are accompanied by synthesis is often overlooked, yet the synthetic activities of the soil organisms are of great significance in determining the availability or supply of certain plant nutrients. The oxidation of any organic compound resolves itself into two downgrade phases, first, the oxidation of the material itself and, second, the oxidation of the microbial cell substance synthesized by the organism. The chemistry of soil organic matter may, therefore, relate more closely to the chemistry of microorganisms than to that of plant residues. If highly available energy material is added to soil, the primary oxidative phase is very rapid with concurrent synthesis of microbial cells; the second phase involving the decomposition of the cell material is slower. Because nitrogen is required for protein synthesis, the nitrogen transformations that accompany synthesis and decomposition can be used within limits as a rough measure of the cell substance present.

The amount of cell tissue synthesized per unit of carbon oxidized is not constant with all types of organism. In general, fungi assimilate and utilize for structural purposes a higher percentage of the substrate carbon than do bacteria, and this group has in consequence a higher demand for nitrogen for protein synthesis. When relatively mature but reasonably available plant residues are decomposed by a mixed flora containing fungi, actinomycetes, and bacteria, there appears to be a demand for about 1.2–1.3 per cent of nitrogen to supply the protein needs of the population that develops. If the nitrogen content of the plant material is less than this figure, the decomposition rate will be reduced because the shortage of nitrogen restricts the size of the microbial population that can develop. In this case additional nitrogen, if supplied in the inorganic form, will be immobilized and converted to microbial protein to the extent of the deficit. On the other hand, if the plant residues have a nitrogen content in excess of the requirement, nitrogen will soon be liberated in the form of ammonia as the amino acids in the proteins are oxidized. The nitrogen requirements in organic matter transformations have often been described in terms of carbon–nitrogen ratios. Plant residues with a carbon–nitrogen ratio initially in the neighborhood of 35:1 contain adequate nitrogen for decomposition. As the available carbohydrates are utilized, the population that can be supported becomes smaller with the result that some of the nitrogen initially incorporated into microbial tissue is no longer required for synthesis of new microbial protein and therefore ammonia begins to appear. As a rough generalization, this may take place when the carbon–nitrogen ratio of the decomposing residues has narrowed to 20:1.

In the decomposition of the more mature low-nitrogen crop residues it can be assumed that most, if not all, of the plant nitrogen is converted to microbial nitrogen. Only when the nitrogen content of the material is initially in excess of 1.5 per cent is there likely to be appreciable release of ammonia by deamination and then only after several weeks. For immediate ammonia liberation, the initial nitrogen content must be in the neighborhood of 2.5 per cent or the carbon–nitrogen ratio about 18:1 (*11*). This is usually only realized by young green plants or leguminous residues.

Although nitrogen is quantitatively the most important plant nutrient the supply of which in the soil is substantially affected by microbial synthesis, other elements also are temporarily immobilized in an organic or inorganic form in the tissues of soil organisms and only become again available to plants on the decomposition of these tissues. Inorganic phosphates may be incorporated in nucleic acids and phospholipids, sulfates in sulfur-containing amino acids and sulfonic esters, minor elements such as boron and manganese may be retained in combinations not at present known. Eventually it may be possible to express quantitatively the requirements of microorganisms for these elements in some such general terms as now can be done for nitrogen. The essential point to be made, however, is that nutrient elements which are required by soil organisms are temporarily immobilized through synthesis, and that this fact must not be overlooked in considering the availability and supply of these elements to the crop. In the case of some elements and in some soils, the amounts so immobilized may be small relative to the supply available to the crop from alternate sources. In other circumstances microbial immobilization may represent an important step in the transformations affecting availability.

THE NITROGEN CYCLE

The nitrogen cycle in soil is almost exclusively a biological cycle. It is not established that there are transformations of importance accomplished by purely chemical means. There is no mineral reserve of nitrogen comparable with that of some other elements, though small amounts of nitrate and ammonia may be added in rainfall. The nitrate supply to the plants depends entirely on the pattern of release of ammonia from organic nitrogen in the soil and, as indicated above, this ordinarily means the release of ammonia from microbial tissues or microbial residues. The nitrification process proper—that is, the oxidation of ammonia through nitrite to nitrate—is entirely secondary in importance. Without ammonia liberation there can be no nitrate.

A major unsolved problem in soil microbiology and biochemistry is why the release of ammonia from the organic nitrogen complexes of microbial origin is so slow. The soil is indeed a "frugal custodian" of nitrogen. It is fortunate for the farmer that this is the case, so that in any one season only a small fraction of the nitrogen reserves becomes available to support the growth of the crop. An acre of prairie soil may contain many thousands of pounds of organic nitrogen, yet the amount becoming available in a single season may be only 150–200 pounds. Various theories have been advanced to account for the relative unavailability of the soil nitrogen. Interactions with lignin residues or with clay colloids have been suggested, yet none seems entirely satisfactory.

In humid regions where leaching occurs, the rate of release—or mineralization—of soil nitrogen may be an important factor in determining crop yields. If at all times there is sufficient nitrogen to meet crop requirements, the soil may be judged well suited for any particular crop. If the pattern of release does not fit the particular nitrogen requirements of the crop for optimum growth, supplementary fertilizer nitrogen may have to be supplied even though the total amount released during the season may well be above the needs of that crop. This may not infrequently be the case with early planted or quickly growing crops. For example, it is not unusual in the corn belt to see nitrogen deficiency symptoms in small grain in the spring or in young corn in a cool, wet period. Such conditions arise because microbial activities are temporarily limited by the low temperature or inadequate aeration.

Rotational systems involving the return of crop residues may considerably alter the pattern of nitrogen mineralization and the amount of nitrogen released during subsequent seasons. More attention has perhaps been given to practices that may supplement the supply of soil nitrogen than to other less obvious steps that, through the agency of the soil population, may affect the seasonal distribution of the nitrogen released. For example, effects on the succeeding crop caused by the incorporation of crop residues may differ according to the time of plowing.

THE PHOSPHORUS AND SULFUR CYCLES

Organic phosphorus transformations in soil, previously largely ignored, are now recognized as being of real significance in relation to the supply of phosphorus to the crop. In some respects the soil phosphorus cycle is far more complicated than the nitrogen cycle (*12*). There is in most soils a substantial reserve of inorganic phosphorus which may be present in one or more of a number of different forms that vary considerably in availability or potential availability. There are, moreover, inorganic reactions that result in the fixation or conversion of soluble phosphates to unavailable forms. The inorganic chemistry of phosphorus and phosphorus compounds in soil is, therefore, of great complexity. Superimposed on this there are the synthetic activities of plants and microorganisms that result in the immobilization of phosphorus in both inorganic and organic combinations. The soluble phosphate utilized by the crop may, therefore, originate in less soluble inorganic sources, or be derived from organic plant or microbial sources if the energy status of the soil is such that mineralization can occur. Because much of the organic phosphorus in microbial tissues is in the form of nucleic acids and phosphoproteins, the immobilization of phosphorus in this form is governed by similar considerations as apply in the case of nitrogen, and there is in fact a quantitative relationship between the nitrogen and phosphorus requirements of microorganisms for protein synthesis. In some plant tissues and notably in cereal crop residues organic phosphorus occurs also in phytin, a compound not believed to be synthesized by microorganisms. Like organic nitrogen, organic phosphorus in soil is less available than might be expected, having in mind the chemical nature of the groupings concerned. Phytin appears to be precipitated in acid or alkaline soils as insoluble phytates of low availability (*1*), though phytin itself when added to soil may be readily and rapidly decomposed. Nucleotides or nucleic acid fragments may enter into some form of combination with the clay colloids so that availability is much reduced.

It has not as yet been found possible to indicate the relationships between carbon and phosphorus that determine whether mineralization or immobilization of phosphorus may occur during the decomposition of crop residues. The phosphorus content of many plant materials is in excess of microbial requirements, but, because inorganic phosphate is normally present in both, no clear picture of the transformations which take place can be obtained. Indeed it is conceivable that the mineralization of nucleic acid phosphorus is controlled primarily by the nitrogen status of the system, and that this phase of the phosphorus cycle may often be subservient to the nitrogen cycle.

Although sulfur is quantitatively far less important nutritionally than phosphorus, there are certain similarities between the phosphorus and sulfur cycles in soil. Like phosphorus, sulfur is involved in organic combination in the proteins of plant and microbial tissues. It also occurs in a variety of thio and sulfonic compounds. Again like phosphorus, sulfur is present in soil also in various inorganic forms, not all immediately available. Sulfur differs from phosphorus in that through the agency of certain autotrophic organisms it may undergo a series of oxidative reactions which terminate in sulfate. In the case of this element, as with nitrogen, the fully oxidized form is available to the plant so that the normal oxidative biological reactions that occur in soil are advantageous in so far as the availability of these elements is concerned. That this is not always the case will be seen later.

OTHER MAJOR AND MINOR NUTRIENT ELEMENTS

Much more is known of the role of soil microorganisms in the nitrogen and phosphorus cycles than in those of some other elements essential for plant growth. Because of the relatively high requirements of crops for these two elements, the transformations accomplished by microorganisms may more frequently determine the available supply and, therefore, the total growth than in the case of elements which do not enter into organic combinations or which are required in lesser amounts.

The problem in the case of elements such as potassium or calcium which have cationic properties and fixed valences is relatively simple. Such elements presumably undergo immobilization by microorganisms when they are present in living tissues and then are not freely available to plants. On death of the tissues they can be immediately reutilized. Even so, there are circumstances in which the supply of such elements may be affected by practices which involve microbiological activity. On certain plots at the Long Ashton Research Station in England, apple trees under clean cultivation develop acute potash deficiency symptoms which do not occur if a grass cover is maintained.

Other cationic elements of variable valency may similarly be incorporated into microbial tissues but in addition may undergo biological oxidations or reductions that may completely change the availability of the element. Manganese is a particularly good example of this group of elements. Exchangeable manganese may be oxidized by heterotrophic organisms to manganic oxide and manganese dioxide (*9*), both of which are presumably unavailable to plants. Little is known of the microbiology of the oxidative process and still less of the reducing conditions that might make oxidized manganese again available. There is some evidence that temporary reduction can be effected by the addition of thiosulfates, which concurrently are biologically oxidized to polythionates and eventually to sulfate (*13*). It is to be noted that this class of transformations is not related quantitatively to the needs of the microbial population in the synthesis of cellular material, as is the case with nitrogen and phosphorus.

Iron also may undergo oxidative or reductive transformations in soil. In this case autotrophic organisms are known which are capable of effecting specifically one oxidative step but iron oxidation is probably not limited to autotrophs.

No doubt it will be found that other micronutrients such as copper, zinc, and molybdenum, whether or not essential to the growth of microorganisms, similarly undergo transformations that are brought about by microorganisms, and which have effects on availability. Indeed, there is already some evidence of this nature in the case of zinc (*3*). Nothing is known as to the nature of transformations that may be undergone in soil by ampholyte elements such as boron, aluminum, and silicon. The available boron has been shown to be correlated with the organic matter content of soils but this is hardly sufficient evidence on which to base a claim that the boron cycle must include steps brought about by microorganisms.

SUMMARY

In attempting to summarize the relationships between the microorganisms of the soil and the availability of the nutrient elements, major and minor, required by the plant, one immediately encounters the difficulty that there is no simple consistent pattern and, therefore, the relationships cannot be neatly defined in a single polysyllable. The soil population is extremely complex. Its activities are not directed towards meeting the needs of the plant. The tempo of its activities is in part controlled by some of the physical factors that determine plant growth but the dominant factor is the energy supply. Although certain changes in the solubility of some plant nutrients may be the resultant of the production of carbon dioxide and other acids, direct effects are of much greater importance. Many plant nutrient elements are required for microbial growth. All oxidation of organic matter is accompanied by the synthesis of microbial tissues with a concurrent demand for essential elements which are utilized and retained, some in organic combination and some only in inorganic forms. The subsequent release of these elements, particularly those such as nitrogen, phosphorus, and sulfur from proteins and nucleic acids, may largely determine the status of the soil with respect to the availability of these elements. Cationic elements having variable valences, though not involved in organic combination and not required in more than trace amounts, may undergo oxidative or reductive transformation by heterotrophic or autotrophic organisms with the result that the availability of the element to plants may be greatly changed. The role of microorganisms in relation to micronutrient supply in soils has not yet been given sufficient consideration.

REFERENCES

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MECHANISM OF ENTRY AND TRANSLOCATION OF MINERAL NUTRIENTS IN PLANTS

CHAPTER

8 The Nature of the Process of Inorganic Solute Accumulation in Roots

T. C. BROYER

THE ECONOMIC aspect of this symposium, sponsored by the University of Wisconsin, widely known for its agricultural research, and by the National Fertilizer Association, is the seeking of information on practices toward adequate production of plant material, for food, shelter, etc., most reasonably under favorable conditions. The mineral nutrition of plants is one phase of a larger field of interest, recognized by the organizers of the symposium, viz., plant–soil interrelations. That part dealing with the soil as a medium for plant growth, is covered in earlier discussions. Similarly, certain parts of the mineral nutrition of plants—for example, essential elements—are presented elsewhere. In addition to information on physico-chemical relations in soil and on the qualitative and quantitative supply of materials therefrom, knowledge of the various aspects of the role which roots play in crop production is important. In particular, therefore, this discussion will present some information now available on the nature of the process of inorganic solute accumulation as well as pose problems for future investigation.*

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*A detailed complete survey of the field is impossible in the limited space of this treatise. Here, a number of important aspects are discussed using illustrative material less frequently summarized [compare Hoagland (*28*)]. The work of several investigators is infrequently and only briefly quoted, since it is anticipated that other authors in this monograph will discuss directly or indirectly in more detail from their own studies and those of their colleagues (e.g., Lundegårdh). Most data presented herein are restricted to observations on roots. However, many important researches on absorption have been performed with other tissues. For these the reader is referred, for example, to the literature by Arisz, Brooks, Osterhout, Robertson, Steward, Stiles, and their associates.

HISTORICAL OBSERVATIONS, DEFINITIONS

Probably the earliest experimentation on this subject was by Woodward (*94*) in 1699. He observed the growth of plants in various types of solution, concluding that "earth, and not water, is the matter that constitutes vegetables." Later, investigators recognized the limitation of this conclusion and each added to the realization that "plants are made of chemical elements obtained from three sources: air, water, and soil; and that the plants grow and increase in size and weight by combining these elements into various plant substances" (*29*). Advancement was attained through the efforts of such men as De Saussure (*22*), Liebig (*40*), Boussingault (*4*), and Sachs (*71*). These physiologists relied as usual on the progress in knowledge afforded by their contemporary physico-chemists. This historical advance in knowledge is ably outlined by Reed (*67*) in *A Short History of the Plant Sciences.*

Absorption and accumulation

Numerous early analyses of plants showed the composition of their various parts. Although not clearly recognized then, it might have been inferred from such data that inorganic solutes were accumulated within the plant from external sources. Probably the first clear evidence of accumulation came from observations of Wodehouse (*93*). Affirmation of inorganic solute accumulation came rapidly through observations of many workers including Cooper and Blinks (*20*), Brooks (*6*), Collander (*16*), Hoagland (*28*), Osterhout (*53*), and their associates. All these studies, for expedience, were performed with algae from marine, fresh, and brackish water. A summary of some of these data is presented in Table I (see *28*, *53*).

Permeability

Similar, specific experimentation on inorganic solute absorption was concurrently and subsequently pursued with other plant materials. Often the results were included under the title of permeability studies. These earlier experiments included the course of absorption with time, effects of external inorganic solute and hydrogen ion concentration, temperature relations, and the like, with isolated blocks of "storage" tissue (52, *81, 82*), epidermal tissue (*24, 34*), whole (intact) plants (57), and roots (*38, 66*). Such results have been reviewed by Brooks and Brooks (*9*) and Stiles (*80*), and in annual reports (*86*) entitled "Permeability" or "Mineral Nutrition of Plants."

The condition of permeability in the strict sense is an important one, for, with a surface limiting entry of materials, the broader aspect of inorganic solute and or water absorption may be controlled or restricted. In the earlier literature the term permeability was broadly used.

TABLE I

Differential Accumulation of Ions in Halicystis, Nitella, and Chara

		Halicystis					
Ion	Sea Water	*Ovalis*	*Osterhoutii*	Pond Water	Nitella	Brackish Water	Chara
	M	M	M	$M \times 10^3$	$M \times 10^3$	$M \times 10^3$	$M \times 10^3$
Cl^-	0.52	0.55	0.60	0.90	90.8	73.0	225.0
Na^+	0.50	0.22	0.56	0.22	10.0	60.0	142.0
K^+	0.01	0.32	0.006	0.05	54.3	1.4	88.0

NOTE: Comparison is made of the concentrations of the principal ions between the vacuolar sap and the external bathing medium under natural conditions.

However, with advance of research, more specific terminology has been applied. Some of these terms have been defined in recent reviews (*10, 11*) and as such will be generally used in this treatise.

It has been recognized for some time that limiting surfaces or membranes are involved where studies on absorption are concerned with living cells and tissues. Several theories have been proposed for the differential permeability of living surfaces relative to penetration of solutes. Studies on the entry of dyes and relatively nonpolar compounds led to the lipoidal theory propounded by Overton (55). This is a solution hypothesis of permeability, assuming as it does that the penetration of different substances runs parallel with their solubility in lipoidal substances of which the limiting surfaces are supposed to be composed. However, there are compounds which are soluble in lipoidal substances, and yet have been found not to enter living cells with significant rapidity. The solution theory which has been modified in numerous ways, is now combined with another concept, that of ultrafiltration through a sievelike nature of the surface. In the latter hypothesis, the rate of migration of a substance into a cell is related entirely to the size of its existing particles or aggregates, the limiting surface serving as an ultrafilter. Early studies of Ruhland (*70*) suggested a close correlation between the rate of movement of nonelectrolytes into cells and their molecular diameter. However, this relation was later reinvestigated by Collander and Bärlund (*19*). Little correlation was found between permeability and molecular size. Lipoid solubility is recognized as a prime factor by Collander; however, he considers it incomplete in so far as it merely indicates what substances will enter cells easily under certain conditions. Although probably important, at least in some cases, ultrafiltration likewise cannot be accepted as a complete theory of cell permeability.

Making use of the colloidal character of protoplasm, Clowes (*15*) has proposed an effect of electrolytes through phase inversion, on cell permeability. Here, it is conceivable that since protoplasm can be regarded as an emulsoid colloidal system, there will be a continuous phase of one composition (dispersion medium) through which is dispersed at least one other phase, the particles of which are discontinuous. It is supposed that changes in permeability are brought about by the continuous and discontinuous phases changing places, that is, by phase inversion. Penetration would take place only through the continuous phase. The hypothesis would be equally applicable to a solution or a chemical combination theory of permeability. This hypothesis in part may be considered suggestive of others included under theories of chemical combination or adsorption. Some investigators (*58, 57, 83*) propose that the substance combines chemically with, or is adsorbed on, a constituent of the protoplasm at its limiting external surface. Absorption would be effected through disturbance of the equilibrium between different protoplasmic constituents, with the result that the chemical or adsorption union is subsequently broken again and the substance is released at other places within, or from other parts of, the cytoplasm. Extension and modification of these concepts have been made by Briggs (*5*), Brooks (*7, 8*), and others (*2, 42, 43, 45, 68*). Some of these investigators visualize a mosaic structure of the protoplasmic surface in space, while Briggs has proposed a structural difference with time (here especially for cation vs. anion absorption). Phase inversion or time change in structure would suggest that permeability changes might be sudden and the delicate balance would in all probability be very easily disturbed so that the whole of the protoplasm would go to one or the other of the conditions. Such a weakness is not inherent in the mosaic structures in space, although the latter may be difficult to envision. The viscosity and electrical charge of the limiting surfaces have been considered by some to be of importance in permeability relations (*74, 42*). Brooks and Brooks (*9*, page 138) quote researches indicating that the rate of penetration of nonelectrolytes into cells is more rapid than that of electrolytes; however, little direct comparison has been made. That permeability to electrolytes relative to the rate of growth is significant and appreciable is evident from the inorganic composition of plant tissues. Any general theory must account for the entry of ions or ion pairs as well as that of acid and basic dyes or compounds of low dissociation in water. Certainly no single permeability hypothesis thus far proposed will account for the penetration of solutes into living cells. Probably several, if not all, of the suggested theories are simultaneously involved in varying degree, depending upon the circumstances within the specific protoplasm concerned and the substance under absorption study. Some detailed reviews have been made by Brooks and Brooks (*9*), Stiles (*80*), and others (*17, 33, 53, 54, 86*).

ROOT ANATOMY

Relatively little research has been made on the relationship between root anatomy and the physiology of absorption. Some early anatomical work was done by Rufz de Lavison (*69*). His observations caused him to consider the endodermis of importance as a limiting surface for the penetration of solutes to the vascular tissues. Several studies were made by Priestley and his associates on the physiological anatomy of roots. Priestley and Tupper-Carey (*65*) suggested the relative impermeability of the growing point of the root to water and substances in aqueous solution. In other publications (*64, 72*), this point was further stressed as well as placing restriction to movement across the root on the differentiation of the endodermis, especially the development of the suberized Casparian strip. A transverse section of a typical root through the transition region of elongation and maturation is reproduced from Priestley (*63*) as Figure 1. Certain features may be particularly observed, namely, the relative areas of cortex and stele, the lack of intercellular spaces between cells and vessels within the stele and their prominence in the cortex, the differentiation of the endodermis and passage cells therein, and the nature of Casparian strip development and the development of root hairs which increase in number in the region of maturation. These anatomical features no doubt play a part in determining the environmental conditions and the rates of processes within the various tissues of roots.

Longitudinally, root structure is distinguished by three portions which grade from one into the other in passing from the tip to the root-stem junction; the extent of each depends upon the relative rates of processes within and differentiation of the component cells and tissues. These three, respectively, have been termed regions of meristematic development, elongation, and maturation. The longitudinal gradation of effectiveness for penetration and movement of materials across tissues is reviewed by Wiersum (*92*). Movement of solutes across the meristematic region and along its walls is possibly restricted by the close packing of cells, the walls of which are reported to be impregnated with protein. Most effective independent flux is considered to occur into the region of elongation. Attended by progressive differentiation in tissues farther from the tip, the rate of transverse independent migration of solutes to the stele is considered to be reduced again. However, in this region of maturation, rates of solute influx to the stele may be modified and increased where root laterals are initiated from within the pericycle, thus effectively "piercing" a possible endodermal barrier. The same general pattern has been pictured by Popesco (*60*). His studies have laid more particular stress on the development of root hairs in the region of maturation. Many investigators have questioned the importance of root hairs in absorption since their pronounced effectiveness is limited to conditions in soils where they may effectively increase the external surface to media of low moisture and/or deficient inorganic solute content.

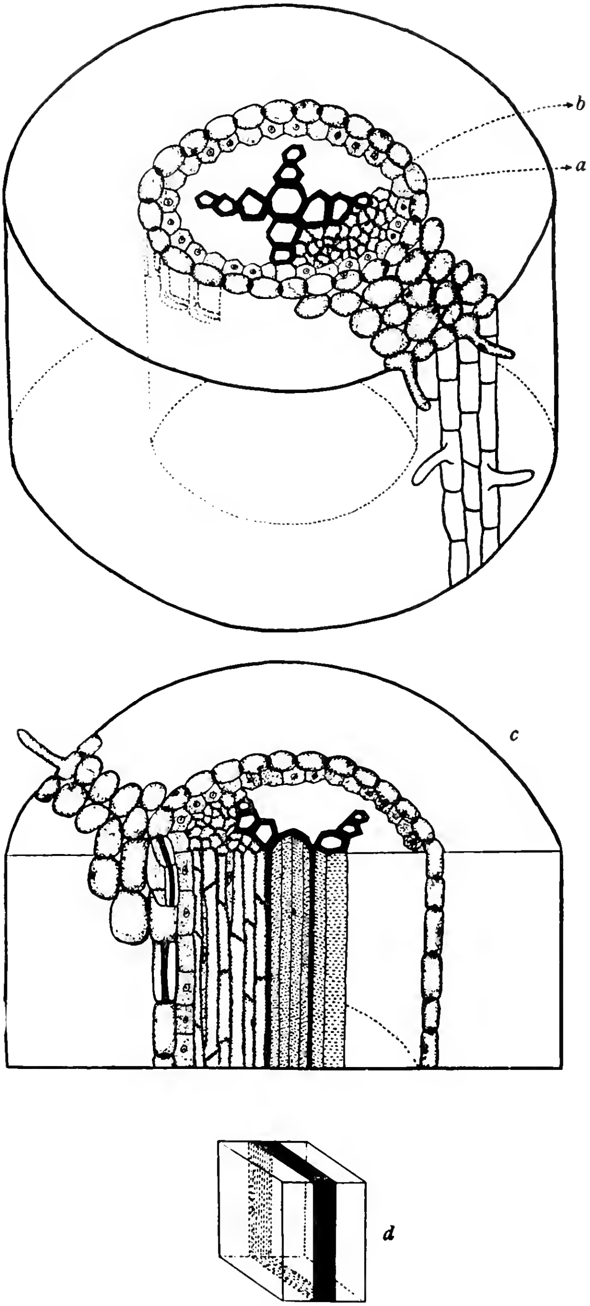

FIGURE 1. Diagram of root structure and endodermis. Transverse section showing (a) endodermis and (b) Casparian strip; (c) median longitudinal section; (d) endodermis with continuous Casparian strip round transverse and radial longitudinal walls.—Priestley *(63)*

A diagram of the spatial relations of different regions in a root tip of tobacco is reproduced from Esau (*23*) as Figure 2. In this figure, the endodermis is indicated only at the levels where Casparian strips are present. The endodermis, as a cell layer, however, becomes defined before the sieve tubes differentiate. This is clearly shown in the upper of two photomicrographs of a root tip by the same author and reproduced here, as Figure 3 (possibly unusual to a certain extent due to a virus infection).

Anatomical features of roots relative to migration and accumulation of inorganic solute have been discussed in detail by Prevot and Steward (*61*). Their studies on distribution of accumulated inorganic solute will be referred to later, but their anatomical diagram is reproduced at this point (Figure 4). The importance of the cortical tissue is emphasized.

MODES OF INORGANIC SOLUTE INFLUX

Several modes of inorganic* solute entry into cells may be distinguished. These are of passive and active nature depending upon whether metabolism of the organism is involved directly or indirectly in the migration (*11, 59, 76, 92*). Four principal means of movement are at present recognized. These include simple and Donnan diffusion, exchange adsorption, and active or metabolic accumulation. These have been schematically represented in Figures 5 and 6 (*11, 92*). Detailed discussions of these mechanisms have been published elsewhere (*2, 11, 42, 53, 54, 76, 92*).

Diffusion and Donnan equilibria

The most obvious mode of migration is that by diffusion. Wherever a difference of concentration (or better, activity) of a solute exists between two regions, there will be a tendency for movement toward the lower values approaching equality of concentration. This mode of flux across the differentially permeable cytoplasm may be very slow, at least with cells of certain species. The net flux of sodium into *Halicystis ovalis* is a case in point (Table I). Obviously, for inorganic solutes, a continuous aqueous medium is essential. It was recognized

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*The movement of organic ions through protoplasm interposed between two solutions needs detailed study.

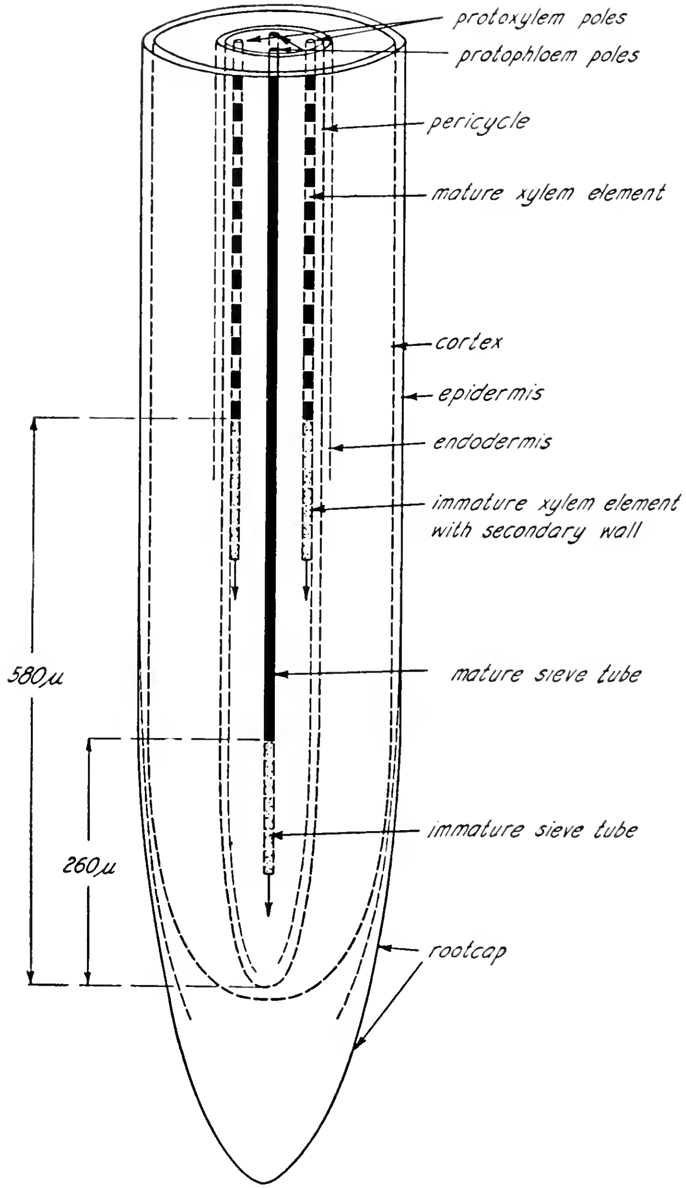


Figure 2. Diagram of a tobacco root tip, showing spatial relations of different regions of the root and of the first vascular elements. This illustrates the order of maturation of tissues in the root tip. In this example the xylem matured at more than twice the distance of the sieve tube from the initials. The endodermis, as a cell layer, becomes defined before the sieve tubes differentiate (see Figure 3A), but develops Casparian strips slightly below the level where the first xylem elements mature. In this figure the endodermis is indicated only at the levels where Casparian strips are present.—Esau *(23)*

FIGURE 3. Longitudinal sections of the apex of a root of curley-top tobacco. Details: *c*, regions of cortical initials; *e*, endodermis; *p*, pericycle; *s*, region of stele initials. A, above, shows spatial order of differentiation of endodermis and phloem relative to the meristem. B, below, shows early differentiation of cortex and stele from meristem initials.—Esau *(23)*

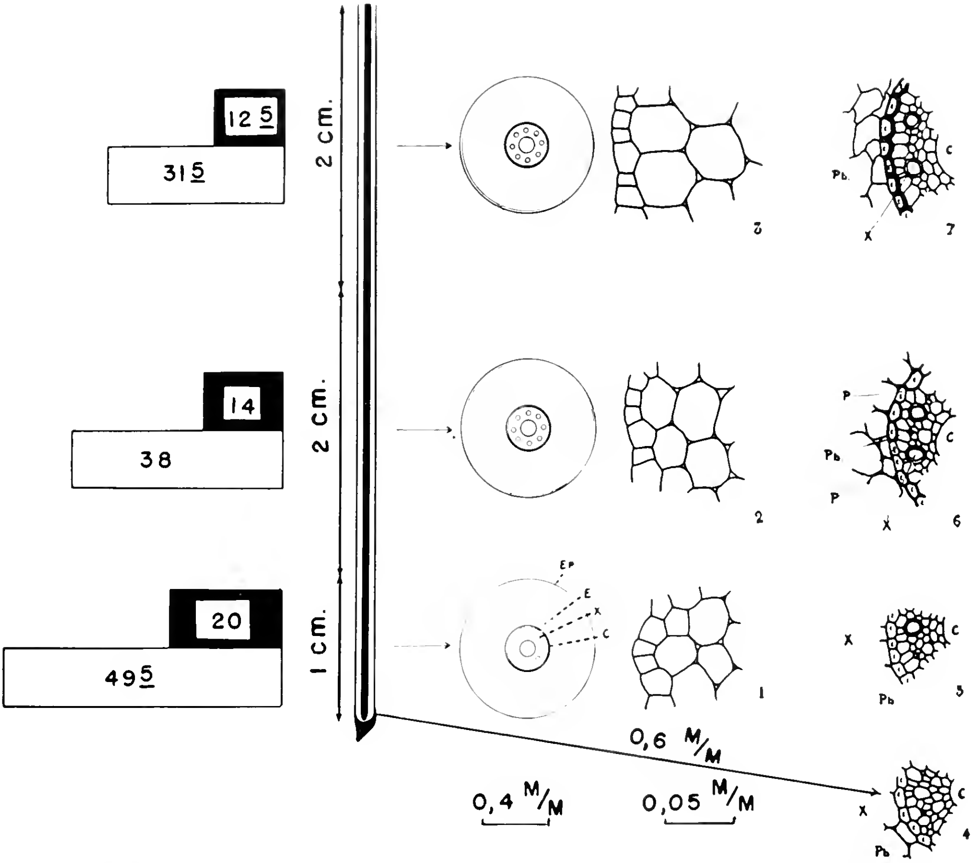


FIGURE 4. Cellular differentiation in median cross sections through 3 parts of barley roots and the relative accumulation of bromide in them. *Right:* Anatomy of barley root ("low-salt" type), at different distances from apex. *Nos. 1–3*, cortex and piliferous layer without suberized exodermis. *No. 4*, 0.6 mm. from apex—differentiation of vascular strands quite evident. *No. 5*, 5 mm. from root apex—unsuberized endodermis. *No. 6*, 1–3 cm. from root apex—endodermis suberized but with passage cells opposite xylem groups. *No. 7*, 3–5 cm. from root apex—endodermis suberized, without passage cells, and, at extremity of segment, also thickened. *Ep*, piliferous layer: *E*, endodermis; *x*, xylem; *Ph*, phloem; *P*, passage cells; *C*, central cavity (axile vessel). *Left:* Absorption of bromide by segments, expressed in m./e. per 1000 gm. fresh weight. External medium, 5 m./e. per L. of KBr.

"Low-salt" roots (white area) from plants 9 days old, grown in distilled water; absorption period 24 hours. "High-salt" roots (blackened area) from plants 15 days old, grown in nutrient solution; absorption period 23 hours.—Prevot and Steward *(61)*

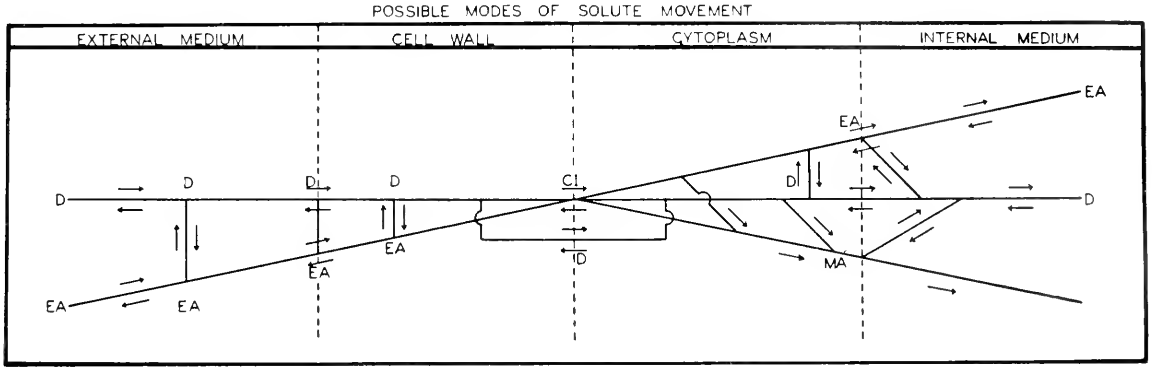


Figure 5. Diagram of some possible modes of solute movement into plants. *D:* Diffusion, simple or Donnan. (1) Low temperature coefficient. (2) Not directly related to oxidative catabolism. Influx limits defined by solute concentration difference, including presence of ions nondiffusible through differentially permeable areas of cytoplasm.

EA: Exchange Adsorption. (1) Low temperature coefficient. (2) Not directly related to oxidative catabolism. Exchange adsorption limits defined by solute concentration difference and extent of colloidal surfaces, dependent on prior or concurrent anobolism.

CI: Cytoplasmic Interaction (including adsorption).

MA: Metabolic Accumulation, associated with living cell. (1) High temperature coefficient. (2) Directly related to oxidative catabolism. Influx limits defined by concentrations of solute and food in cytoplasm, and rate of oxidative catabolism.

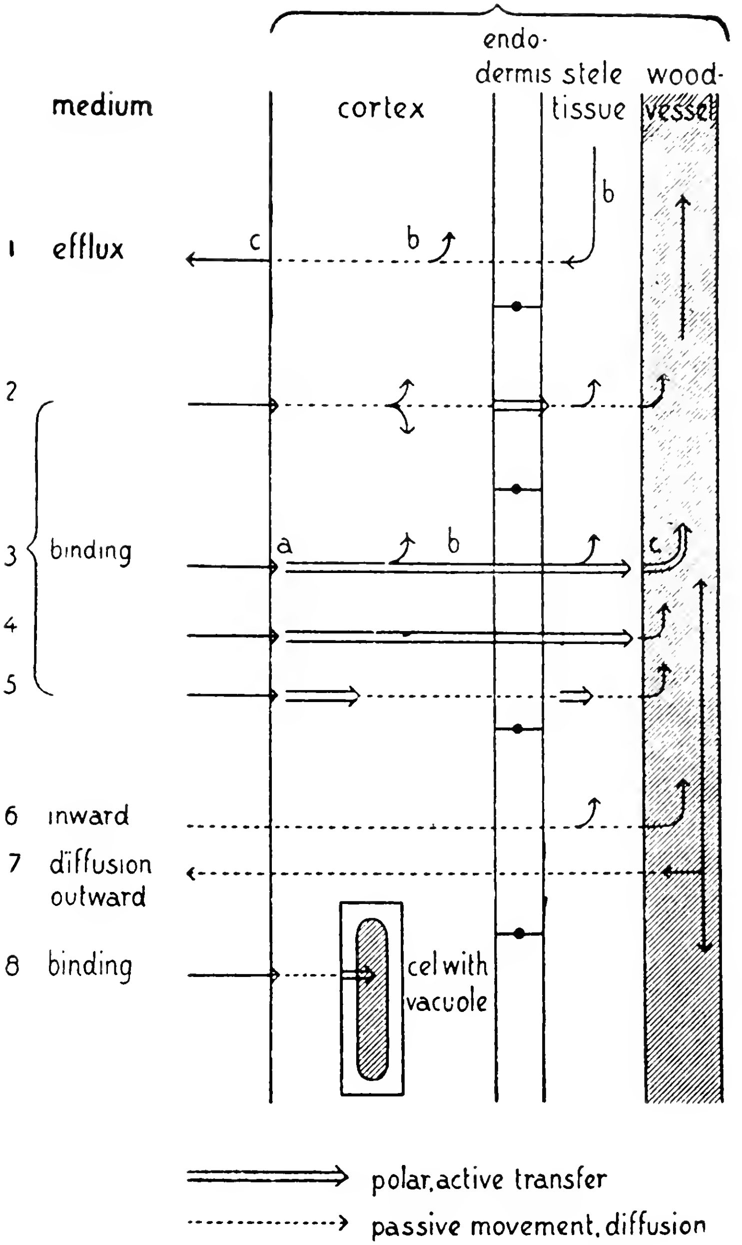


FIGURE 6. Scheme illustrating the theoretical considerations concerning lateral transport through living root tissues.—Wiersum *(92)*

early by Donnan that under certain circumstances a modified equilibrium of diffusion may obtain. This phenomenon occurs where one of the ion pairs of a compound in solution does not readily penetrate a differentially permeable membrane interposed between two aqueous solutions. The equilibrium conditions imposed by this phenomenon are such that, for monovalent ions for example, the product of the molar concentrations of the cation (C^+) and anion (A^-) pairs in the one medium equal the same product in the solution on the opposing side of the limiting surface, i.e., $(C^+)_i \times (A^-)_i = (C^+)_e \times (A^-)_e$. Expressed otherwise, at equilibrium the ratio of the concentrations of the diffusible cations in the two media should equal the inverse ratio of the diffusible anions, i.e., $(C^+)_i/(C^+)_e = (A^-)_e/(A^-)_i$. Studies in vitro have shown this phenomenon to be qualitatively valid; however, in nature the ratios required at equilibrium are not usually observable. The latter observations indicate that although generally applicable, ionic equilibria of this sort are not universally attained in systems with living cells. Compare the relevant inverse ratios of cation to cation and anion to anion, particularly for K^+, Na^+, and Cl^-, for *Halicystis, Nitella,* and *Chara* in Table I. One must infer, therefore, that the Donnan equilibrium as originally proposed is not to be regarded as of primary importance between at least some living cells and their natural environment (*11*, *54*).

Exchange adsorption

With living tissues, we are dealing with colloidal systems, and the phenomenon of exchange adsorption can play a role in cellular physiology of inorganic solute absorption. Ions are adsorbed on the surfaces of charged colloids. These may be exchanged for ions with the same sign from the dispersion medium. This mode of ionic migration has been extended recently to include an exchange of ions between colloidal micelles where interpenetrating spheres of ion oscillation exist. Thus, also, the movement of ions along surfaces, without intermediate passage into the dispersion medium, may be effected. These exchange phenomena are schematically represented in Figure 7, from Jenny and Overstreet (*36*, *37*). Such movements are discussed in detail elsewhere in this book.

Active or metabolic accumulation

Simple diffusion leads toward an equilibrium in which a substance in solution tends to approach equality of concentration within the system. Under appropriate conditions, Donnan phenomena and/or exchange adsorption may lead toward a condition in which differential accumulation of inorganic solutes may obtain within cells. However, some evidence would suggest that these modes of migration are not all inclusive. A complementary process has been proposed and recognized (*11*, 27, *28*, *42*, *59*, 75, *86*), whereby inorganic solutes at least are accumulated within cells of plants. This mode of movement is considered to be directly related to metabolism of the cell and is a polar or "unidirectional" migration. Each and all of these modes of accumulation, induced as well as active, are probably involved in variable degrees in the net movement into cells and tissues of plants. In this regard, roots of higher plants are of particular concern initially, since they are the organs concerned primarily with absorption for these species.

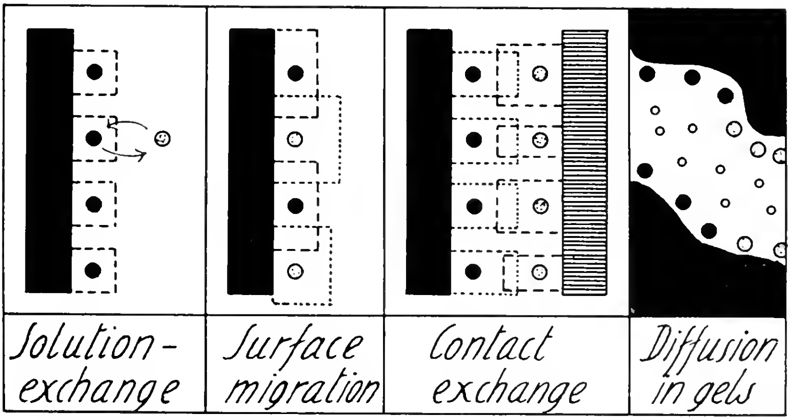


FIGURE 7. Diagram of various modes of ionic migration, especially adsorption exchange.—Jenny and Overstreet (*37*)

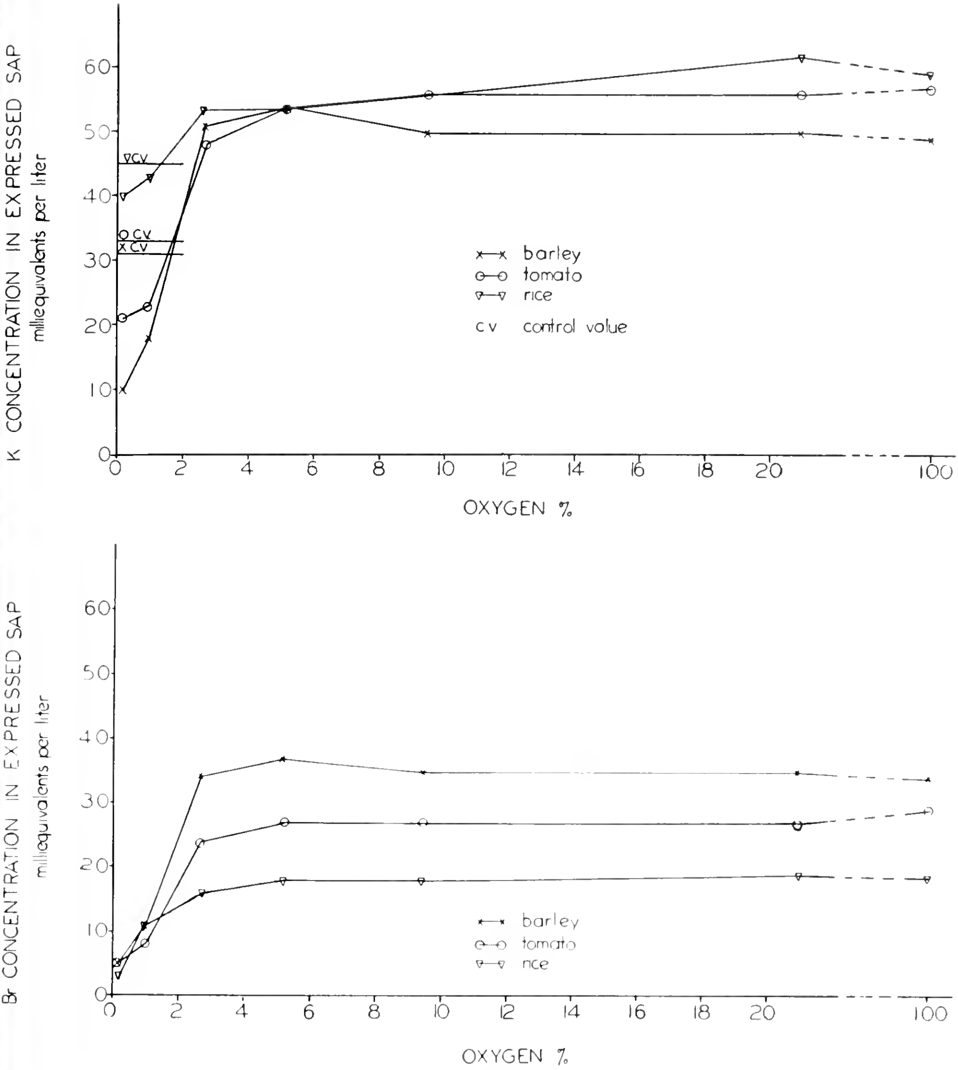
FACTORS DETERMINING RATE AND EXTENT OF INORGANIC SOLUTE ABSORPTION

Aeration

There are two main groups of influences which determine the rate and extent of solute absorption. These factors may be due either to external or environmental conditions, or to internal controlling conditions. Of the external or environmental conditions, aeration, temperature, and the supply of water and mineral salts are important. These conditions may alter and determine the internal physico-chemical conditions and the relative rates of processes, some interrelated with absorption, in roots. Studies with abscised roots of barley (*30*) and oats (*59*), and roots of decapitated wheat plants (*42*) have clearly shown the necessity of an aerobic environment for the accumulation of salt in such organs. Experimental results with abscised roots of tomato and rice, as well as barley, are presented in Figures 8A and 8B, from experiments of Vlamis and Davis (*88*). Here, the reduced accumulation of potassium and bromide at oxygen values below 3–5 per cent is clear. A fairly close correlation exists between these effects of oxygen supply on accumulation, and on total respiration by comparable tissues. This is evident when the respiration curves shown in Figure 9 are compared with the accumulation curves of Figures 8A and 8B. Similar concordance between respiration and ion accumulation rates in potato roots has been reported (*77*). The relationship between these factors will be discussed further in another section.

Temperature

The temperature of the environment is a second factor which may control the rate of physico-chemical processes. As with aeration effects, a similar correspondence between the effects of various temperatures on the processes of accumulation and respiration have been obtained. Several investigators have reported these phenomena with roots (*30, 42, 84, 91*). Such effects may be exemplified by data derived from experiments of Ulrich (*84*) with abscised roots (Figure 10). In these histograms, it may be observed that increases in both accumulation and respiration rates are associated with rise in temperature of the medium bathing the tissues. More than doubled increases in rates for a ten-degree rise in temperature are indicative of these processes being of physico-chemical nature. From the concordance of temperature effects obtained between accumulation of inorganic solutes and respiration (especially the enhanced respiration), investigators have generally inferred some interrelation between them. This is quite possible, since



FIGURES 8A AND 8B. Effect of aeration on salt accumulation by abscised roots of barley. Plants 4 weeks old; abscised roots in experimental culture solution (KBr, 5 milliequivalents per liter) for 24 hours. *Above:* potassium absorption; *Below:* bromide absorption.—Vlamis and Davis *(88)*

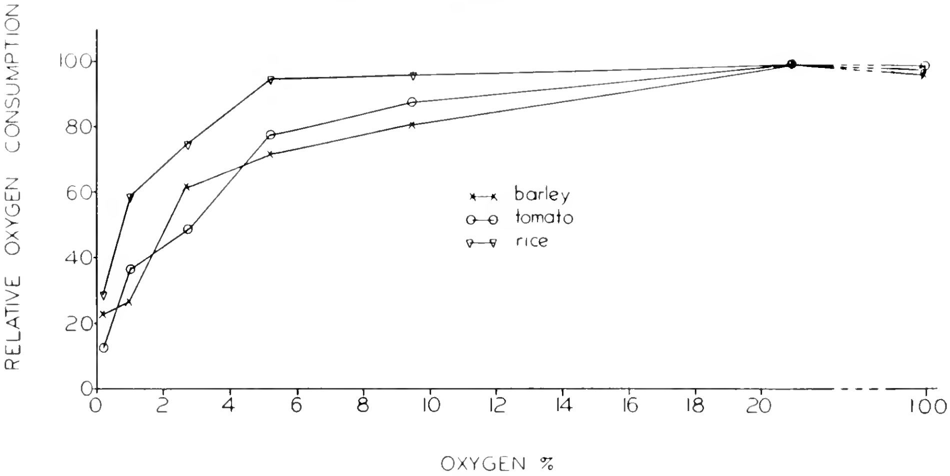


FIGURE 9. Effect of aeration on respiration by abscised roots of barley. Conditions of growth and experiment as in Figures 8A and 8B.—Vlamis and Davis *(88)*

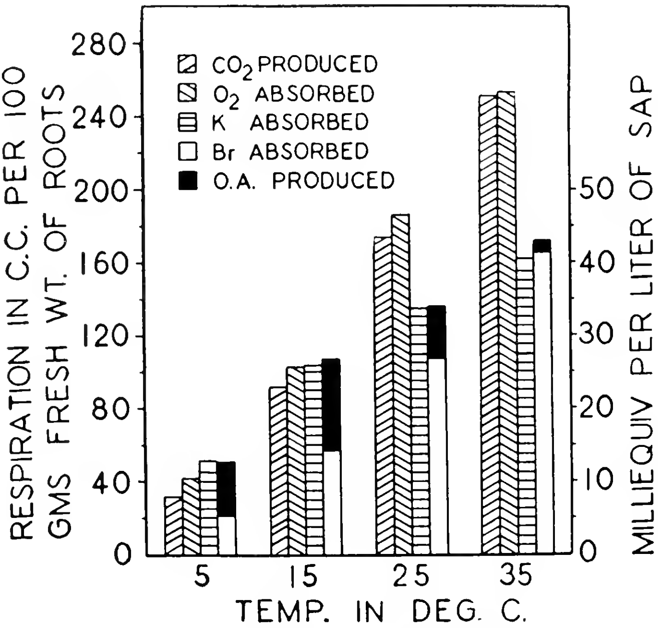


FIGURE 10. Effect of temperature of the bathing medium on inorganic solute absorption and respiration by abscised barley roots. Plant growth, 5½ days, dark, in a dilute artificial tap water. Experimental solution 0.005*M* KBr + 0.0005 *M* $CaSO_4$; time, 8 hours.—Ulrich *(84)*

the Q_{10} values for both absorption and respiration are very similar in magnitude for like increases of 10° C. These vary from 2 to 3 at lower temperature intervals to about 1.5 at the higher. The respiratory quotient does not vary significantly over the temperature range studied; similar Q_{10} values are obtained for both oxygen involved and carbon dioxide evolved. The Q_{10} values for absorption of bromide are consistently above those for potassium over similar temperature ranges, the difference decreasing with increase of temperature. If any significant inward migration of potassium by exchange over that between anions is possible, this should tend to bring the Q_{10} values for potassium, compared with bromide, closer together (see later discussion on "anion" respiration).

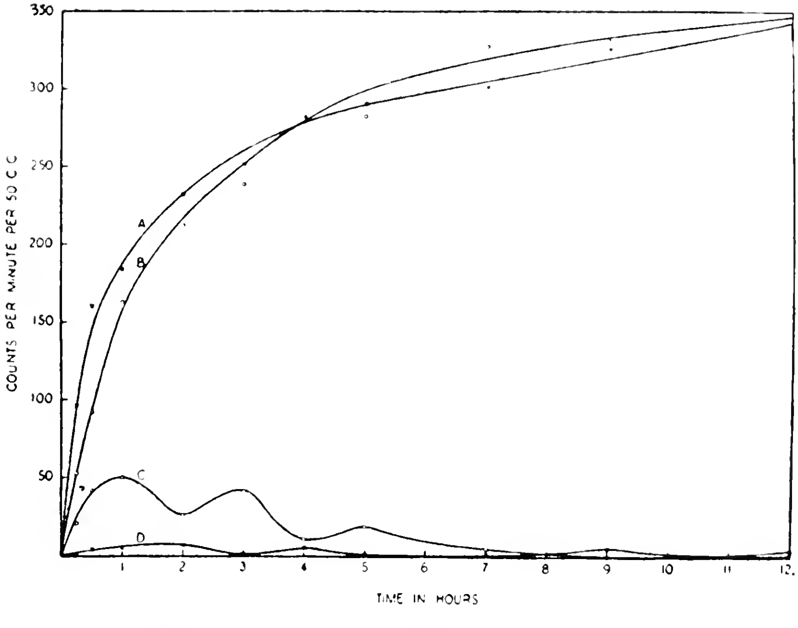


FIGURE 11. Effects of temperature and nature of the culture medium on the rate of outward movement of radioactive potassium from abscised barley roots which had previously accumulated radioactive potassium. Plants 4 weeks old. *Set A:* 0.005*N* nonradioactive KCl at 20.0° C.; *set B:* 0.005*N* nonradioactive KCl at 1.0° C.; *set C:* distilled water at 1.0° C.; *set D:* distilled water at 20.0° C.

In the immediately preceding discussion, the pronounced effects of the external conditions of aeration and temperature on the over-all process of salt accumulation were considered. Aside from the possible influences of aeration and temperature upon permeability itself, it would be expected that the temperature condition might also modify the rates of diffusion and exchange. Effects of this sort would be manifested through increase of kinetic energy of mobile substances in solution. Since the energy intensity for migration of a solute would be proportional to the absolute temperature, this influence, however, would be relatively small.

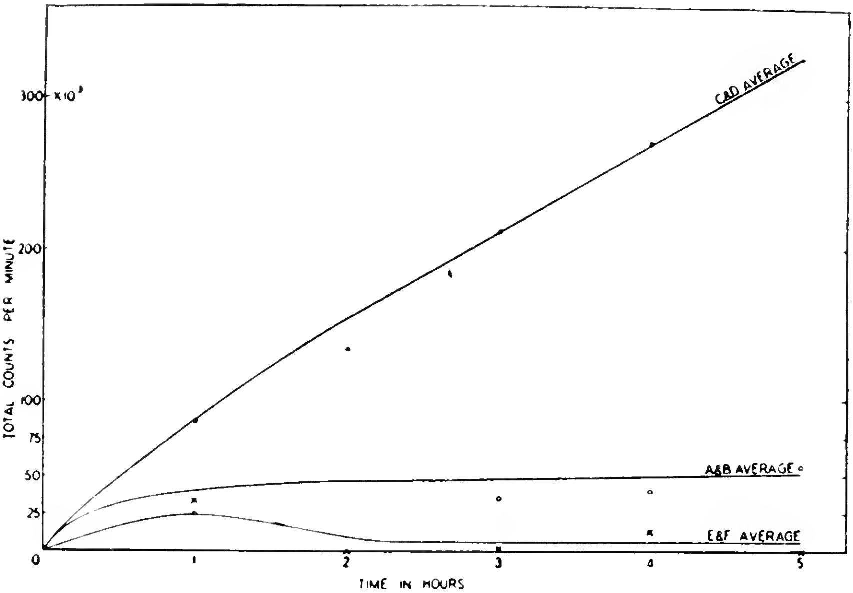


Figure 12A. Effects of temperature and aerobic nature of the culture medium on the rate of inward movement of radioactive potassium. Barley plants 3 weeks old. *Sets A and B:* 0.5° C., CO$_2$-free air passed through the culture solution; *sets C and D:* 20.5° C., CO$_2$-free air passed through the culture solution; *sets E and F:* 20.5° C., CO$_2$-free N$_2$ gas passed through the culture solution. The experimental points indicate radioactivity measurements of K*Cl culture solutions in which excised roots were immersed. The data are plotted as differences between the initial culture value and the values at subsequent periods of time.

A study was made (*13*) of the possible effects of temperature and aeration on exchange adsorption with roots. The results of these studies are summarized in Figures 11, 12A, and 12B. In Figure 11, the rates of outward isotopic diffusion and exchange of potassium at two temperatures are shown. Simple diffusion of salt from roots into distilled water was very slow; exchange of cations was rapid. About 10 per cent of the previously absorbed radioactive isotope of potassium was readily exchangeable (compare with data of Overstreet and Jacobson, referred to later for other cations and for anions). The rates of ionic exchange were not significantly influenced by temperature. In Figure 12, a similar experiment on exchange at two aeration levels is presented. The upper graph (12A) shows the relative accumulation of radioactive potassium

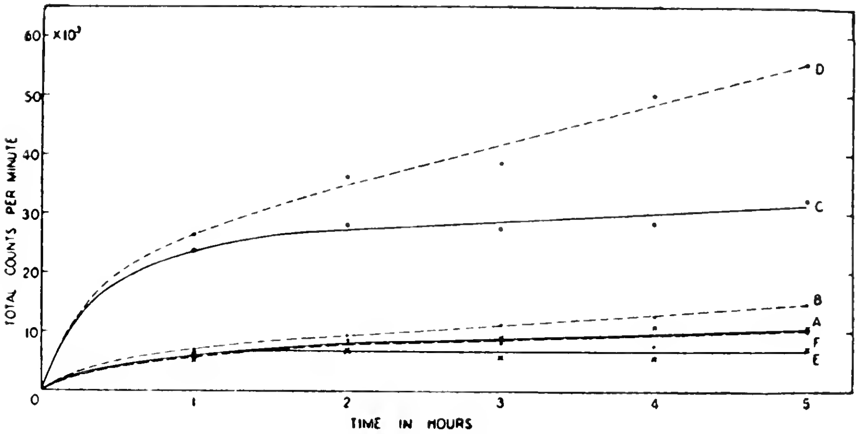


FIGURE 12B. Effects of aerobic and anaerobic culture medium environments on the rate of outward movement of radioactive potassium. Following the duplicate treatments presented under Figure 12A, individual sets of excised roots were exposed to culture solutions of 0.005*N* KCl at 20.5° C. as follows:

Pretreatment—Absorption of K*Cl: *A–B*, temperature 0.5° C., air-treated culture; *C–D*, temperature 20.5° C., air-treated culture; *E–F*, temperature 20.5° C., N_2-gas–treated culture.

Treatment during efflux study: *A*, CO_2-free air passed through culture solution; *B*, CO_2-free N_2 gas passed through culture solution; *C*, CO_2-free air passed through culture solution; *D*, CO_2-free N_2 gas passed through culture solution; *E*, CO_2-free air passed through culture solution; *F*, CO_2-free N_2 gas passed through culture solution.

during pretreatments with two conditions of temperature and aeration. The lower graph (12B) shows the experimental measurements of isotopic exchange under aerobic and anaerobic conditions. The exchange rate was not enhanced by aeration (compare with upper graph). On the contrary, an even lower rate of exchange was obtained with aeration, which is probably related to concurrent reaccumulation by metabolic influx or internal translocation of previously adsorbed ions from the effective loci of exchange. That exchange does occur is clear from these experiments. It should be mentioned, in passing, that while this limited exchange of isotopic cations was proceeding, a pronounced net influx of total potassium occurred where oxidative metabolism was favored (i.e., with aeration and at higher temperatures). These observations suggest two modes of movement of inorganic solute apart from diffusion phenomena, namely, an ionic exchange, more or less independent of metabolism, and an accumulation mechanism, the latter directly related to concurrent metabolic activity.

Water supply

A supply of water to the living system is obviously necessary. However, it may be less obvious that the relative supply may alter or determine the rate of internal processes, especially the accumulation of inorganic solutes. The influence is indirect. The movement of water into tissues will dilute the concentration of solutes within the system, thus lowering the accumulation level therein. Since the maximum inorganic solute level of tissues is limited by hereditary potentialities and rates of processes, further movement of such solutes inward may occur through dilution within, or by translocation from, the loci of initial absorption and accumulation to other parts of the plant (30). (Compare with the discussion of internal solute concentration.) If, as is considered to be the case by most investigators, the movement of inorganic compounds is in the form of ions or ion pairs, water is obviously essential to this dissociation. Certainly ion exchange phenomena are of this sort. The water factor in mineral nutrition is discussed elsewhere in this monograph by Biddulph, Burström, and Wadleigh.

Inorganic solute supply

The supply of mineral salts is axiomatic. Furthermore, the concentration and relative proportions of such substances may alter or control internal processes. Again, the inorganic solute status may modify the rates of the two processes earlier referred to, namely, accumulation and respiration. The effect to be noted is that of concentration of a single salt in the bathing medium on the rates of respiration and of accumulation of the ions of the compound supplied. With increasing

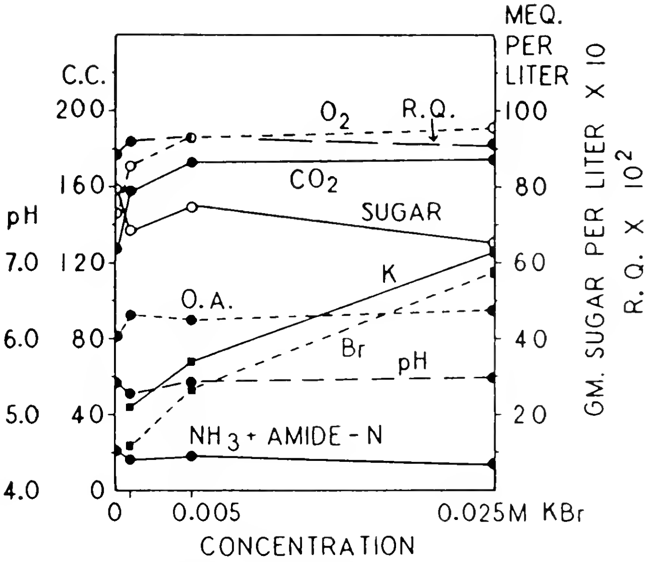


Figure 13. Effects of potassium bromide concentration on the metabolic activity of excised barley roots maintained at 25.0° C. for 8 hours. K and Br values given as milliequivalents per liter of expressed sap. R.Q. = CO_2/O_2; Sugar—total sugars expressed as grams glucose per liter of expressed sap. The following are given as milliequivalents per liter of sap: O.A. (total nonvolatile organic acids) and NH_3 + amide-N (ammonia plus amide nitrogen). pH values were determined on expressed sap. Conditions of growth and experiment were the same as for Figure 10, except that KBr only was supplied in the experimental culture solution.—Ulrich *(85)*

concentration of a dilute salt solution, the rate of accumulation is likewise greater. A corresponding, though not necessarily proportional, increase in respiration is generally observed. These effects are graphically represented in Figures 13 and 14 from Ulrich (*85*); see also Figure 15. In Figure 13 it may be observed that the rates of accumulation of the ions of a particular ion pair supplied (here, potassium and bromide) may be rather similarly affected. However, the relative rates of anion and cation accumulation may be distinctly different. Thus, a monovalent ion (cation or anion of a pair) usually accumulates more rapidly than a divalent "partner." This may be seen by comparing the absorption of bromide and calcium from calcium bromide or potassium and sulfate from potassium sulfate shown in Table II (*84*). Further, the rate of absorption of a particular ion is markedly influenced by the nature of the accompanying ion of opposite charge. Thus, bromide

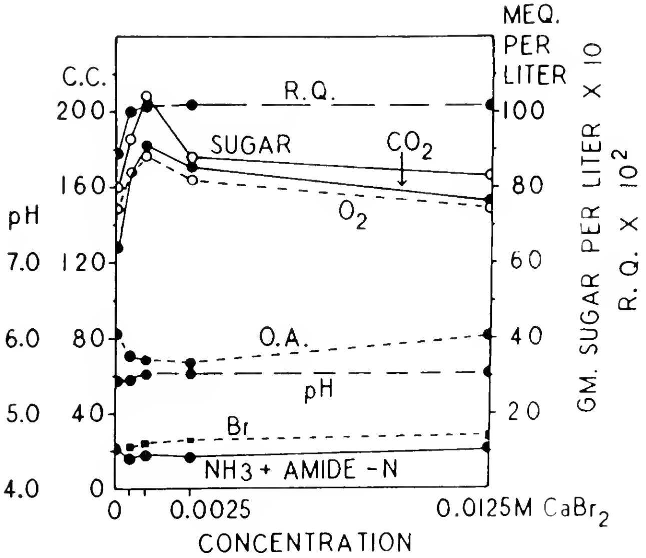


FIGURE 14. Effects of calcium bromide concentration on the metabolic activity of excised barley roots maintained at 25.0° C. for eight hours. Data expressed in the same way as in Figure 13. Conditions of growth and experiment were the same as for Figure 10, except that $CaBr_2$ only was supplied in the experimental culture solution.—Ulrich (*85*)

TABLE II

Effects of Absorption of Various Salts by Excised Barley Roots*

Culture Solution	Total CO_2 Produced, cc.	O_2 Absorbed, cc.	R.Q., CO_2/O_2	Total Sugars, g./l.	pH	Composition of Expressed Sap: Total O.A., m.e./l.	m.e./l. Absorbed Cation	m.e./l. Absorbed Anion	Excess Cation over Anion	O.A. Change	NH_4-N + $CONH_2$-N, m.e./l.
None (Original roots)	—	—	—	10.6	5.23	37.9	—	—	—	—	7.8
0.0005 *M* $CaSO_4$	128.0	145.7	0.88	8.0	5.42	40.7	—‡	—‡	—	—	10.6
0.003 *M* $CaSO_4$	130.9	145.2	0.90	9.7	5.38	40.7	—‡	—‡	—	—	9.2
0.005 *M* KBr 0.0005 *M* $CaSO_4$	173.5	186.2	0.93	7.5	5.44	45.1	33.7	26.7	+7.0	+7.2	9.3
0.0025 *M* K_2SO_4 0.0005 *M* $CaSO_4$	134.4	154.2	0.87	8.0	5.33	53.1	20.5	—‡	+20.5	+15.2	9.1
0.005 *M* KH_2PO_4 0.0005 *M* $CaSO_4$	128.0	166.9	0.77	7.1	5.30	61.8	24.2	—‡	+24.2	+23.9	9.7
0.00167 *M* K citrate 0.0005 *M* $CaSO_4$	147.4	188.4	0.78	6.1	5.69	74.5	40.6	—	+40.6	+36.6	6.8
0.005 *M* $KHCO_3$ 0.0005 *M* $CaSO_4$	41.8†	181.1	0.23	6.6	5.84	84.5	50.7	—	+50.7	+46.6	7.8
0.005 *M* KNO_3 0.0005 *M* $CaSO_4$	191.6	201.8	0.95	6.1	5.68	57.2	51.8	28.4	+23.5	+19.3	10.7
0.0025 *M* $(NH_4)_2SO_4$ 0.0005 *M* $CaSO_4$	202.2	229.1	0.88	6.9	5.42	46.6	20.9	—‡	+20.9	+8.7	28.9
0.0025 *M* $CaBr_2$ 0.0005 *M* $CaSO_4$	170.5	163.7	1.04	8.8	5.53	33.2	—‡	12.9	−12.9	−4.8	8.2
0.0025 *M* $Ca(NO_3)_2$ 0.0005 *M* $CaSO_4$	216.4	195.8	1.11	7.3	5.77	37.2	—‡	18.2	−18.2	−0.2	14.7

*One hundred grams of excised roots were placed in 3 liters of culture solution maintained at 25° C. for eight hours. Average of 10 values for original roots, 7 values for KBr-treated roots, and 2 values each for KNO_3 and $CaBr_2$ treatments.

†CO_2 as $KHCO_3$ present initially in culture solution 336.0 cc.; CO_2 present in final culture solution 168.0 cc; CO_2 in alkali tower 209.8 cc.: $(168.0 + 209.8) - 336 = 41.8$.

‡No significant change as indicated by culture-solution analyses.

NOTE: Conditions of growth and experiment same as for Figure 13. Temperature 25.0° C., for 8 hours.—Ulrich (*84*) usually migrates more rapidly into roots when supplied with potassium than with calcium. This is shown in Figure 15 from Hoagland (*27*) (compare Table II and Figures 13 and 14). The relative rates of migration between cations or between anions will no doubt be modified by the relative importance of the various modes of movement in any particular case.

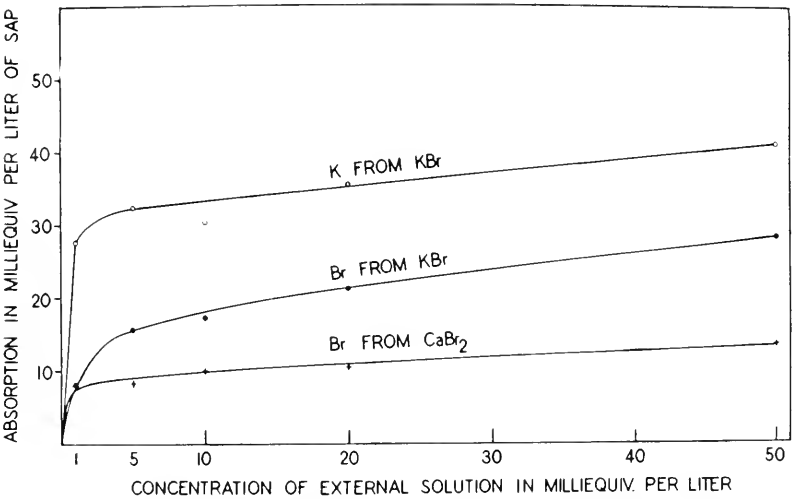


FIGURE 15. Effect of concentration of the culture medium on the absorption of K and Br from KBr and $CaBr_2$ solutions by abscised barley roots. barley plants grew in one-half strength (standard) nutrient (22 days). Absorption period 8½ hours; culture solution temperature 16.5° C.; concentrations from 1 to 50 milliequivalents per liter.—Hoagland *(27)*

Where more than one inorganic solute is supplied in the medium around the roots, the rates of ion absorption are further complicated. Early studies with intact plants have shown the movements of one cation to be modified (usually reduced) by the presence of another cation (*26*). Similar effects have been obtained between cations, between anions, and between ions of opposite charge (the latter referred to previously for single inorganic solutes). These results recall to mind early studies on antagonism (*53, 80*). The latter, however, were related to amelioration of toxic effects of single inorganic solutes supplied in high concentrations through addition (usually of low concentrations) of another salt. The interionic effects observed by Hoagland (26) were concerned with low concentrations throughout, usual to natural conditions in soils. Such effects, where more than a single inorganic solute is present in the external medium, are not always of a sort to suggest interionic lowering of energy intensity of constituent ions in the external medium nor decreased permeability of the membrane, since reductions in absorption of an indicator ion are not always obtained. On the contrary, under appropriate conditions, the presence of a complementary salt may lead to increased inward migration of the primary inorganic solute ions. Addition of polyvalent cations in appropriate concentrations to a single indicator salt medium may increase the rate of entry of both cation and anion of the inorganic solute in question over the absorption rate from the single component salt solution alone. Figure 16, from Viets (87), shows the increased migration rate of potassium and bromide into roots when calcium bromide is supplied in addition to potassium bromide in the external medium. Similar data for radioactive bromide only are shown in Table III. In addition, these latter data indicate corresponding increases between the indicator anions absorbed and respiration. It may be noted in passing, that this polyvalent cation effect is not observable under environmental conditions which inhibit oxidative metabolism (87). These results suggest that

TABLE III

Effect of a Divalent Cation on Accumulation of Salt and on Respiration by Excised Barley Roots

Experimental Conditions	Br*	Accumulation Ratio	Respiration Rate O_2	Respiration Rate CO_2
	counts/min./set		mm.^{3}/hr.	
H_2O	0		34.0	33.5
KBr^* 0.005 N	334	4.2	37.5	37.0
KBr^* 0.005 N + $CaSO_4$ 0.015 N	430	5.7	42.0	41.0
External medium	83			

NOTE: Plants grew in darkness for five days in dilute calcium sulfate solution. Experimental period 8 hours; temperature 20° C.

the primary effect may be either on the plasma membrane or on a "surface" metabolism intimately related to the permeability of protoplasm.

Symbiosis

There is one other relationship between inorganic solute supply and accumulation, which is restricted to certain types of plants. A symbiotic association is recognized between parasites and roots of certain plants. First, there is the association between certain bacteria and the roots of legumes. This association is ultimately concerned with the accumulation of nitrogen in these species. The activities of these organisms relative to nitrogen fixation are related to the supply of nitrogen in the external medium bathing the roots. Discussion of these interrelations is beyond the scope of this review, but they should be recognized in the general nitrogen economy of such plants. Again, there is a symbiotic association between the roots of host pine species and parasitic fungi with the development of mycorrhizae. This association has been

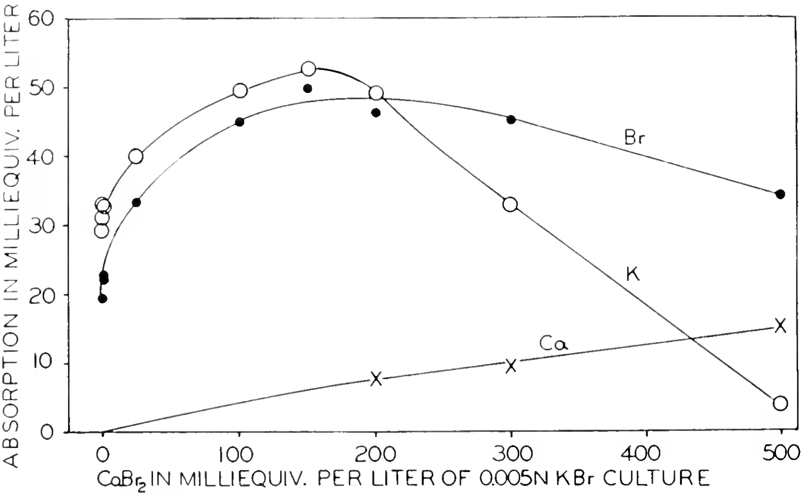


FIGURE 16. Effect of a divalent cation in the culture solution on the accumulation of KBr by abscised barley roots. Plants 4 weeks old; growth in diluted nutrient solution. Experimental period 8¼ hours; temperature 20° C.—Viets *(87)*

studied with relation to inorganic solute absorption and the concomitant respiratory activity. It has been shown that mycorrhizal roots exhibit a greater rate of respiration than comparable nonmycorrhizal organs. A higher level of inorganic solute accumulation is attained where this symbiotic association exists. Whether the increased rate of inward migration of inorganic solute is related to a greater surface exposure to the root medium, to increased permeability, or to a differential metabolic rate has not been established (*39*).

Hereditary limitations

In addition to the external or environmental conditions, there are internal controlling conditions which may determine the rates and extent of inorganic solute absorption. These two groups are not wholly mutually exclusive, but the latter are dependent upon the former in many cases. Thus, the factors of aeration and temperature, for example, while applicable to discussion as external conditions altering rates of processes, also alter the internal conditions where, perhaps, they may be more aptly recognized as effective.

Of particular concern are the hereditary potentialities of the individual which are predetermined by its ancestry. These potentialities limit and determine the physico-chemical conditions and the extent to which internal processes may proceed. The specific structure, implying the molecular structure and arrangement in protoplasm, with all its inherent potentialities, is relatively constant for each elementary species. This concept is strikingly exemplified in the differential movement of inorganic solute ions and their accumulation within specific organisms. Thus, although exposed to like external conditions, two distinct genetic types of plant may exhibit markedly different permeabilities to, and/or net influx levels of, inorganic solutes. In Table I, it may be seen that *Halicystis ovalis* and *Osterhoutii* display salt concentrations in their vacuolar fluids which are quite distinct one from the other, each characteristic of the species under their similar natural conditions. Environmental conditions may alter internal conditions and processes within an organism, but the extent of the effect will be limited by its genetic potentialities. Thus, in one experimental study, Collander (*18*) exposed the roots of various species of plants to a common, yet perhaps unnatu-

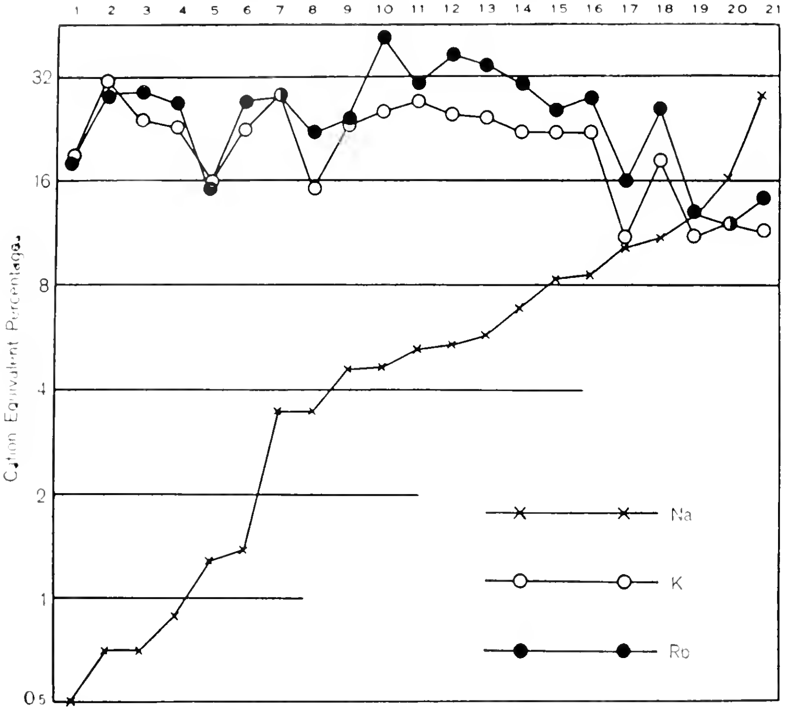


FIGURE 17. Differential accumulation of cations by various species of plants. Equivalent percentages of Na, K, and Rb in plants containing these cations in equivalent amounts. The plants are arranged according to increasing Na content.

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*

The plants were cultivated in a solution: 2 $NaNO_3$ + 2 KH_2PO_4 + 2 RbCl + 2 $MgSO_4$ + 2 $Ca(NO_3)_2$ + 0.01 $MnCl_2$ + an unknown amount of Sr. In preparing the solution, 0.0005 milligram equivalents $SrCl_2$ per liter of solution was intentionally added; the Ca salt used probably contained some Sr. The figures preceding the chemical formulas give the milligram equivalents per liter of solution.—Collander *(18)* ral, bathing medium. He observed, under comparable external conditions, the relative distribution of three monovalent cations in these plants. It was found that the relative accumulation of sodium varied markedly between the various types of plants. These data are presented in Figure 17. The inference which may be drawn from these results is that the sodium concentration was determined not only by the environmental conditions, internal conditions, and rates of processes but limited in extent also by the hereditary potentialities of each species.

Previous history of tissues

Further, what a plant may do at any time under a particular set of conditions is preconditioned by both hereditary and physiological factors. Its genetic potentialities are preconditioned by the phylogenetic history of the species. The physiological preconditioning is related to what the plant did during its past ontogenetic development. Thus, current observations of processes in plants are dependent upon the previous history of its tissues. If, for example, excised roots are low in salt, further solute may migrate inward very rapidly during a subsequent interval of time. With such low-salt tissues, the rate of salt absorption may be as rapid for an excised root system as for entire plants. If, however, roots have accumulated inorganic solutes in the past, under favorable environmental conditions approaching upper limits imposed by their hereditary potentialities, they may be restricted, under such circumstances, from further accumulation. Such high-salt excised roots are close to their dynamic equilibrium relative to inorganic solute concentration. Further inorganic solute movement into these organs will depend upon some disturbance of internal conditions. If the organ, by some means, (see discussion of external water supply) is reduced in its inorganic solute concentration, further solute can then be absorbed under appropriate conditions. Translocation of inorganic solutes from root to shoot may accomplish this end. Data from such studies (*30*) are reproduced in Table IV.

Continued accumulation of inorganic solute is dependent on another internal condition, the supply of adequate amounts and type of substrate for oxidative metabolism. The substance of importance in this regard is sugar. During inorganic solute accumulation, there is a concomitant increased loss of sugar which is reflected in the enhanced respiratory activity of roots. If the supply of this material should be or become low, the accumulation of inorganic solutes may be restricted. A part of the limited ability of high-salt roots to accumulate inorganic solutes may be related to this factor. It has been shown further that such roots are usually what may be referred to as high-salt, low-sugar systems. The latter factor is of secondary importance in the experiments reported in Table IV. The substrate limitation can be more clearly divorced from the high-salt factor by depletion of the carbohydrate in root systems, through prolonged aeration of excised roots. When low-salt roots are so treated, further accumulation is limited unless sugar is concurrently supplied externally during the experimental study. Although less effective than an inherent internal supply, an external application of sugar will correct a substrate limitation to some degree (Table V). Usually, this accentuated restriction is rather unnatural, but the necessity of a ready substrate for the processes of respiration and inorganic solute accumulation is emphasized. As will be pointed out later, the respiratory quotient in roots is frequently approximately one,

TABLE IV

Effect of Salt Status on Further Salt Absorption by Barley

Experimental Conditions	Expt. A		Expt. B	
	Absorbed from solution		Absorbed from solution	
	K	Br	K	NO_3
	m.e./liter		m.e./liter	
High-salt excised roots . . .	−0.20	0.50	0.67	0.89
Low-salt excised roots . . .	2.66	1.90	3.02	2.93
High-salt entire plants . . .			2.05	2.40
Low-salt entire plants . . .			3.66	3.49

NOTE: Plants 3 weeks old. Nutrient solution during growth renewed daily for high-salt sets and not renewed for low-salt sets. Composition of culture for experiment A: 0.0075 *M* KBr initially; experimental time 20 hours at 25° C. Composition of culture for experiment B: 0.0025 *M* $Ca(NO_3)_2$; 0.005 *M* KNO_3; 0.001 *M* $MgSO_4$; and 0.0005 *M* KH_2PO_4, initially; experimental period 7 hours.

suggesting the utilization of sugars primarily in these regards. Furthermore, subsequent to depletion of sugars, respiration in roots (measured by the carbon dioxide evolved) may proceed without marked reduction in rate, although the respiratory quotient undoubtedly changes (Figure 18). However, concomitant inorganic solute accumulation appears to be greatly reduced (*30*), indicating that other substrates are probably less effective, indirectly, for this process. Similar results have been reported elsewhere (*42, 43*).

TABLE V

Effects of Sugar Supplied to Culture Medium on Accumulation of Potassium and Nitrate

Preliminary Treatment	Treatment During Absorption Period	Absorbed from Solution		Sugar in Sap	
		K	NO_3	Initial	Final
		m.e./liter		g./liter	
	Experiment A				
1. Aerated in tap H_2O	aerated	0.87	1.61	2.3	0.3
2. Aerated in tap H_2O + glucose	aerated	1.97	2.57	7.2	1.5
3. Aerated in tap H_2O	aerated plus glucose	1.74	3.46	2.3	0.8
3 days	*27 hours*				
	Experiment B				
1. No treatment	aerated	1.71	2.05	15.1	2.5
2. Aerated in tap H_2O	aerated	0.86	0.80	2.3	0.3
3. Aerated in tap H_2O	aerated plus glucose	1.53	3.32	2.3	0.2
4 days	*27 hours*				

Concentration of K in Sap, Experiment B
(Initial conc. of K in sap approx. 25)

	m.e./l.
1.	93.6
2.	63.0
3.	89.6

Permeability of roots

A very important aspect of internal control is posed by the permeability of roots. The rate and levels of accumulation of inorganic solutes are governed physico-chemically by two prime factors, the gradient of energy intensity of the substances accumulated, between the internal and external phases of the system, and the permeability of the cells or tissues. These two factors have been discussed at some length in recent publications (*9, 11*). Permeability is obviously particularly concerned with phase boundaries. The permeability is related to the specific protoplasmic organization characteristic of the species and altered, within limits imposed by genetic factors, by the environment. Some of the features of surface limitations have been outlined earlier. A lowering of temperature reduces the rate of metabolism, but it will also increase viscosity. Permeability may be more readily separated from metabolic effects on accumulation, by studies in which the external medium is generally more concentrated than that within the tissues, so that simple diffusion could account for the influx without neces-

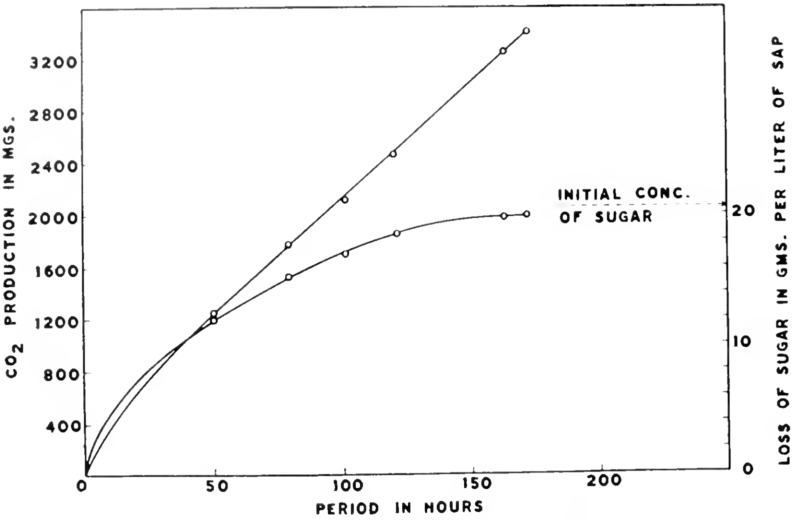


FIGURE 18. Time-course of respiration (CO_2 evolved) and sugar depletion in abscised barley roots.

sarily involving other modes of movement. From data such as those presented in Table VI (*32*), the marked reduction of the inward migration rate for potassium bromide with lowered temperature is clear. The pronounced effect of deficient aeration in reducing the rate of accumulation of inorganic solute from solutions of low concentration

TABLE VI

Effects of Aeration, Temperature, and Cyanide Treatments on Salt Movement into Excised Roots of Barley

Experimental Conditions	Net Absorption K	Net Absorption Br	Respiration Rate
	m.e./l. sap		mg. CO_2/ g./hr.
Experiment A			
H_2O, air	−1.6	0	0.29
H_2O, N_2	−5.1	0	0.16
KBr 5 m.e., air	29.1	22.3	0.32
KBr 5 m.e., N_2	−0.1	3.2	0.12
KBr 5 m.e. + KCN 1 m.e., air	−2.5	0	0.19
KBr 50 m.e. + KCN 1 m.e., air	4.2	6.8	—
Control roots (initial status)	24	0	
Experiment B			
KBr 60 m.e., 0.75° C., air	18.5	16.1	
KBr 60 m.e., 0.75° C., N_2	5.8	9.7	
KBr 60 m.e., 18.5° C., air	58.4	50.0	
KBr 60 m.e., 18.5° C., N_2	7.2	10.7	
Control roots (initial status)	22.0	0	

NOTE: Experiment A: plants 3–4 weeks old; experimental period 8 hours, temperature about 20° C. Experiment B: plants 3 weeks old; experimental period 12 hours, temperature 18.5° C.

was pointed out under discussion of environmental factors and exemplified by the data in Figures 8A and 8B. A similar result is shown with corresponding treatments in Table VI where the data also show the greatly decreased rate of penetration of potassium bromide into roots subjected to lowered temperature where diffusion may account for the movement. Studies of this sort have been extended to include the influences of certain respiratory inhibitors. Cyanide treatment, for example, results in effects which suggest an induced anaerobiosis. Here, a reduced influx of inorganic solute occurs, similar to the effect of nitrogen in replacing the usual oxygen contents of external bathing media. This may be due either to a reduced permeability of protoplasm or to a decreased, inwardly directed, gradient of energy intensity of the inorganic solute. Passage of carbon dioxide gas through the root bathing medium likewise results in restricted influx. Here, the effect is probably twofold. Oxygen concentrations in the medium and within the roots are probably reduced to adverse levels, and a tendency toward reversal or inhibition of the usual respiratory reactions is created. Deleterious effects of carbon dioxide are evident not only on the accumulation of inorganic solute from dilute solutions (*31*) but also on influx, where movement is with the direction in which the concentration of the solute decreases. Relatively prolonged treatment with this gas is quite destructive to the organization of protoplasm, and an early reduced influx is followed by an increase indicative of permanent injury. Thus, while the effects of low temperature, nitrogen, and cyanide are usually quite reversible, treatments with carbon dioxide above approximately 20 per cent in air frequently lead to irreparable damage to the cells. In one experiment (Figures 19 and 20) with decapitated plants, the stumps were subjected to suction. The rate of exudation was suppressed with either nitrogen or carbon dioxide applications to the roots. This indicates a reduced permeability to water or some secondary effect, possibly associated with a modified concomitant flux of solute. At the same time, the rate of bromide movement into and through the system was decreased. Metabolic accumulation of inorganic salt was simultaneously arrested (*32*). Where carbon dioxide was used, the later rise in this curve suggests a destruction of the usual differential permeability of the tissues, with the drawing of solution through the roots as though through an open conduit.

Hydrogen ion concentration

Studies have shown an effect of the change of hydrogen ion concentration on the structure of proteinaceous colloidal systems. Any marked shift of pH in protoplasm would probably lead to a deranged cytoplasmic organization. However, in living tissues, a tendency to-

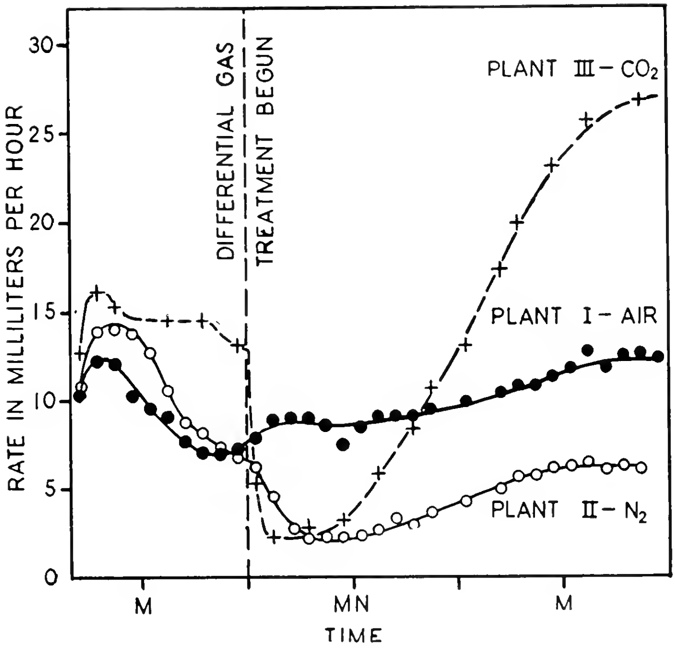


FIGURE 19. Effects of differential gas treatments on the rate of exudation by decapitated tomato plants. Tomato plants grew with aeration, in a culture solution of the following composition: KNO_3, 0.0025*M;* $Ca(NO_3)_2$, 0.0025*M;* $MgSO_4$, 0.001*M;* KH_2PO_4, 0.0005*M;* Fe as iron tartrate (1 ml. of 0.5 per cent solution per liter of culture solution). Micronutrients as required. Growth period, 47 days. Experimental culture solution, KBr, 5 milliequivalents per liter. Gases were continuously passed through the solutions. All roots were aerated until 6 P. M. Differential gas treatments of air, N_2, and CO_2 were then applied. Suction equal to 740 mm. Hg was continuously applied (except during observations on volume of exudate and during removal of increments of exuded fluid) throughout the experiment. At the termination of the experiment the abilities of the plants to develop an exudation pressure (as a measure of the state of the root systems) were noted. The following results were obtained: aerated cultures, appreciable and maintained exudation pressure; N_2-treated cultures, a weak yet maintained pressure; CO_2-treated cultures, no exudation pressure over an extended interval of time. The roots in the CO_2-treated culture were flaccid at the termination of suction exudation.

ward modification of the hydrogen ion concentration by a moderate alteration of the pH of the external medium is counteracted within the protoplasm by direct buffer action of constituent compounds and, possibly, by a similarly effective modification in the rates of some metabolic reactions. Thus, the pH in cells is maintained within rather narrow limits (3, 78). If any marked change of internal hydrogen ion concentration did occur, no doubt accumulation, respiration, and other processes would be strikingly modified. Since no marked change of internal conditions occurs with the alteration of external hydrogen ion conditions, this factor was not discussed under external influences with relation to inorganic solute absorption. That this latter process is not significantly modified by such an external influence has been shown

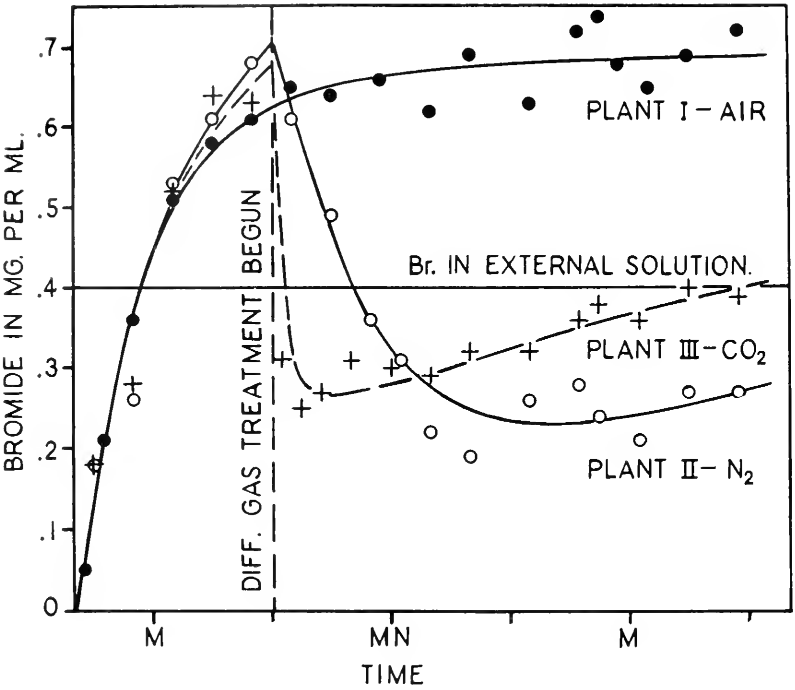


FIGURE 20. Effects of differential gas treatments on the concentration of bromide ions in exudates from decapitated tomato plants. Data same as in Figure 19.

in several studies (*31, 45;* compare *91*). Data from one such experiment are reproduced in Table VII (*31*). Here, the pH values of exudates from decapitated plants show remarkable constancy even though the hydrogen ion concentration of the medium bathing the roots varied over a wide range. The accumulation of inorganic solute which was indicated by potassium and bromide concentrations in the exudates was not appreciably different. This point will be referred to later in another connection.

TABLE VII

pH Effects on Concentration of Potassium and Bromine in Exudate from Barley Seedlings

pH External Solution Initial	pH External Solution Final	pH Exudate	Concentration in Exudate K	Concentration in Exudate Br
			m.e./l.	
3.5	4.8	5.3	27.6	27.4
6.0	5.9	5.4	29.8	37.3
8.1	7.7	5.3	32.9	42.2

NOTE: Initial concentration in exudate — K = 12.5; initial concentration external solution = 5; exudate collected during period of 6 hours.

BIOCHEMICAL ASPECTS—ENERGETICS AND MECHANISM

Growth and metabolic level

Steward (75, 76) has drawn attention to a possible correlation between salt accumulation by cells and the growth and metabolism thereof. His chart of the relative rates of net movement of inorganic solute into various types of plant cells and tissues is reproduced as Figure 21. The favorable situation of roots may be seen, showing marked salt accumulation under favorable external and internal conditions. Here, also, are summarized the effects of previous history, particularly physiological preconditioning on further accumulation of salt. Steward also stresses renewal or continuance of growth as requisite for further primary or active absorption (compare Lundegårdh, *46*). In certain of his own studies with storage tissues, he has obtained data which he feels suggest a correlation between protein formation and

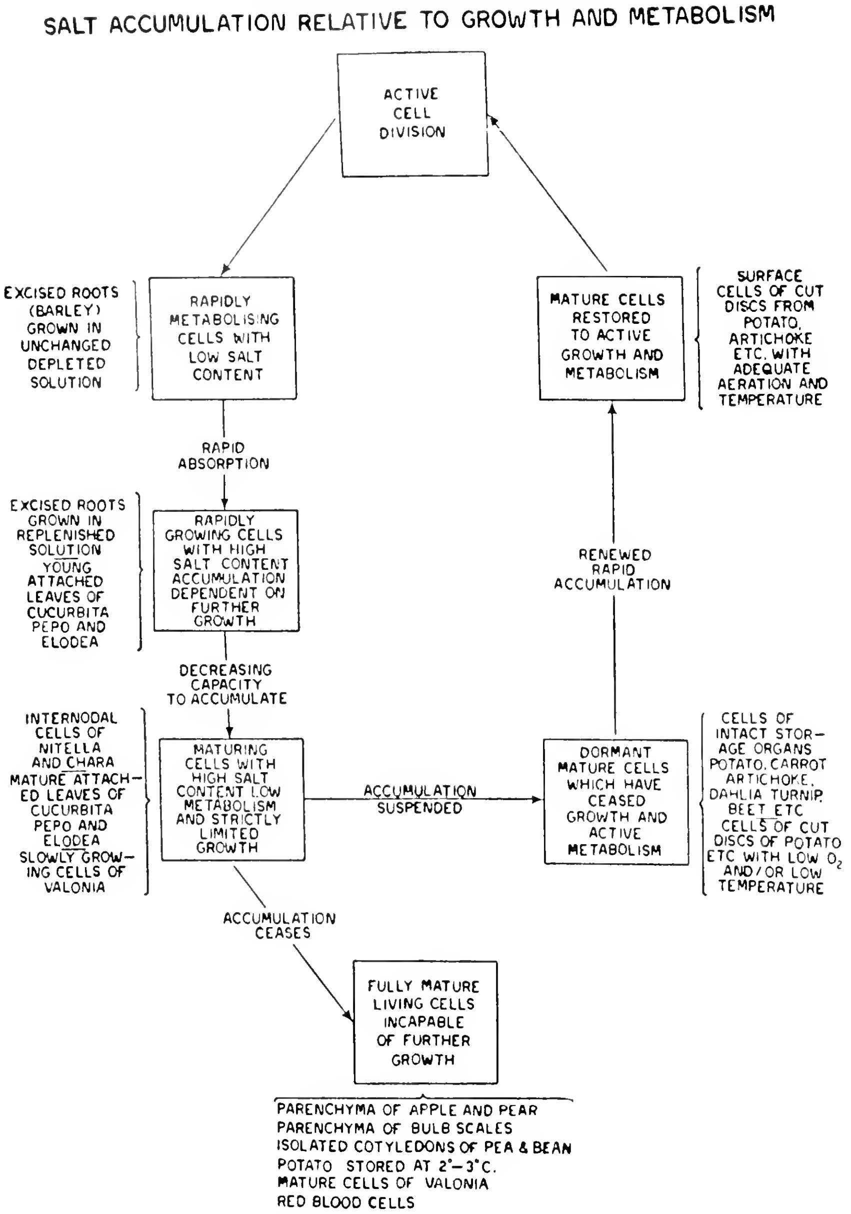


FIGURE 21. Classification of representative types of plant tissues and cells with regard to their ability to accumulate inorganic solutes.—Steward *(75)*

continued accumulation. Such did not seem to be significant in short time studies with excised barley roots, where amino and amide nitrogen in sap were measured (*84, 85*). A general correlation has been shown for various types of tissues between the accumulation of inorganic solute and their general metabolic level as measured by respiratory activity. This relation is shown in Table VIII (*16*). Certainly, high absorption or accumulation rates for inorganic solute must, in some way, require the expenditure of energy by cells. A causal relationship has been sought, therefore, between the enhanced respiration in the presence of a salt supply and the accumulation of inorganic solute in cells.

TABLE VIII

Relationship Between Respiration Intensity and Salt Accumulation by Certain Cells from Various Plant Species.—Collander (*16*)

Object	Temperature	Mg. O_2 wt. per hr. per g. Fresh	Accumulation
Barley roots	25° C.	0.30	rapid
Potato disks	23	ca. 0.15	less rapid
Elodea leaves	23–25	0.135	less rapid
Tolypellopsis	23–25	0.06–0.08	—
Apple disks	23	0.048*	very slow
Chara	23–25	0.02–0.04	—
Valonia	?	ca. 0.0001	very slow

*Calculated from the CO_2 liberated

Organic acids and respiratory quotient

In certain cases of metabolic accumulation, an approximate equivalence in movement of anion and cation into roots is observed. However, this is not generally the rule from single salt media. More frequently there is an excess migration of one ion over the other, apart from certain limited ionic exchanges. When this occurs, there is evidently an adjustment within the plant such that ionic equivalence therein is maintained. Organic acids appear to play a role in this regard. When the rate of cation influx is more rapid than anion, organic acid anions increase in concentration within the cell, balancing the excess cation. An internal decrease in organic acid anions accompanies an excess inward migration of anions. The balancing effect of organic acids is often reflected in the process of respiration of the cells; for example, the ratio of carbon dioxide evolved to oxygen involved is increased in accord with a metabolic respiration of organic acids. These relationships are shown in Table II from Ulrich (*84*).

Respiration and accumulation

Several mechanisms for active or metabolic inorganic solute accumulation have been suggested. Some seem untenable, while others do not, at least with present knowledge, seem to explain all the various observations. The suggestion of chemical union between cations and some protoplasmic constituent, associated with a pronounced hydrogen ion gradient, does not appear to be in accord with observations from studies with various pH values of the external medium (compare (*31,78*) and (*53,54*)). The proposal that all accumulation of ions is an exchange phenomenon seems to be incomplete. Ionic exchange, through or between solutions or by surface migration, is a real part of the overall process of inorganic solute movement, but does not appear to explain the major metabolic accumulation. Various anions and cations are produced metabolically which might exchange for solute ions from the external medium, yet their production to even high concentrations within cells does not necessarily lead to an inward migration and accumulation of inorganic solute. Briggs (*5*) and Brooks (*7, 8*) have been strong proponents of this hypothesis (compare *13*). All investigators recognized at least a partial role in the movement of inorganic solute by diffusion, Donnan, and exchange phenomena. Most of them propose, in addition, some mechanism whereby energy can be expended by the cell through oxidative metabolism and applied toward accumulation and retention of solute. The suggested cause and effect relationship between the enhanced respiration and metabolic accumulation of inorganic solute, has been pointed out earlier.

Observations of net solute influx, from measurements on internal and external media of cell or integrated systems, indicate an over-all migration of inorganic solute from a phase of lower to a phase of higher solute activity.* The required energy expenditure is possibly related to the oxidative formation of high energy specific protoplasmic constituents, or special configurations thereof, in which form they interact with inorganic solute ions at outer loci within the protoplasm. Through this association, the activity of the solute constituent is effectively lowered from that in the external medium. The nature of the binding is a moot question. Whether secretion is then involved as one step from protoplasm to lumen is debatable. It seems more reasonable to the writer to postulate that the higher solute activity is resident within the intermicellar fluid of the inner regions of protoplasm, the inward flux therefrom to the lumen occurring with the direction in which the effective concentration of the individual solute constituent decreases. Rather then, subsequent translocation of the complex (by diffusion and/or protoplasmic streaming) to loci more internal within the protoplasm, where conditions and rates of related processes differ from those existing nearer the exterior surface, may there involve the rupture of the solute-protoplasmic union through reduction, rearrangement, or deformation of the protoplasmic constituents resulting in the release of the accompanying inorganic member to the intermicellar fluid of the colloidal system. Concurrently, the protoplasmic constituent of the complex is transformed from a higher energy content to one of lower energy, the energy being transferred to the solute constituent associated with its higher activity, where compared with that in the external phase. Thus, in brief, the solute migrates from regions of protoplasm where the ability to hold solute constituents is great to regions where the retention is critically lessened, concomitant with a gradient of decreasing oxidative metabolism (*21*). The polarized flux of solute, therefore, parallels specific protoplasmic connection and disconnection with solute constituents characteristically accumulated by the particular plant in accord with its genetic constitution. The energy expended toward the configuration or formation of the protoplasmic cycle is transferred in essence to the solute as a net result with an increase of the internal solute activity by means of this intermediate interaction between

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*Rates of concurrent diffusion and/or exchange adsorption are limited by the permeability of the interposed protoplasm and the activity or concentration gradient (cf. Dean and Overstreet in this book) of the solute constituents.

protoplasm and the migrating constituent. Labile vacuolar solute of individual cells may be retransported where the external supply becomes limiting. Under these conditions, the activity gradient of the solute constituent is reversed between the aqueous phases of the lumen and protoplasm and renewed movement to other loci in the symplast may occur (*1, 2, 3, 21, 32, 35, 42, 43, 68, 92*).

The effects of a reduced rate of oxidative metabolism (attained by reduction of either the aerobic or temperature level of the external medium and internal tissue system) on inorganic solute accumulation were discussed earlier and are exemplified in Figures 8A, 8B, and 10, and in Table VI. The effect of an impaired rate of oxidative metabolism on inorganic solute accumulation has also been studied through the use of respiratory inhibitors. The effect of cyanide in the external bathing medium of roots on potassium and bromide migration is shown in Table VI, from Hoagland and Broyer (*32*). Here, comparison may be made between the effects of reduced aeration and the application of cyanide. These treatments similarly lead to a marked reduction in accumulation of inorganic solute, while at the same time the usually enhanced respiration is inhibited. The cyanide impairment of accumulation and respiration is like an induced anaerobiosis. Similar observations have been made by Lundegårdh (*43, 44*), Machlis (*49*), and Milthorpe and Robertson (*51*). Lundegårdh (*46*) and Machlis have observed inhibition of enhanced respiration as well as accumulation of inorganic solute where other steps in the respiratory cycle are blocked (Figure 22). Interference with glycolysis and either the dehydrogenase or oxidase systems may inhibit inorganic solute accumulation. It should be noted in passing that with cyanide a concomitant reduction in permeability *per se* is possible. This is similar to effects produced with nitrogen gas, where relatively high concentrations of potassium bromide are applied. The reduced accumulation of inorganic solute by cyanide treatment has led Lundegårdh (*43, 44*) to propose that the energy transfer to absorption is concerned particularly with the terminal oxidase system of roots, especially the cytochrome-cytochrome oxidase system. His detailed theory of the accumulation-respiration process (*42, 43*) concerns primarily the anion of an inorganic solute. He considers the energy expended to be restricted to the migration of anions

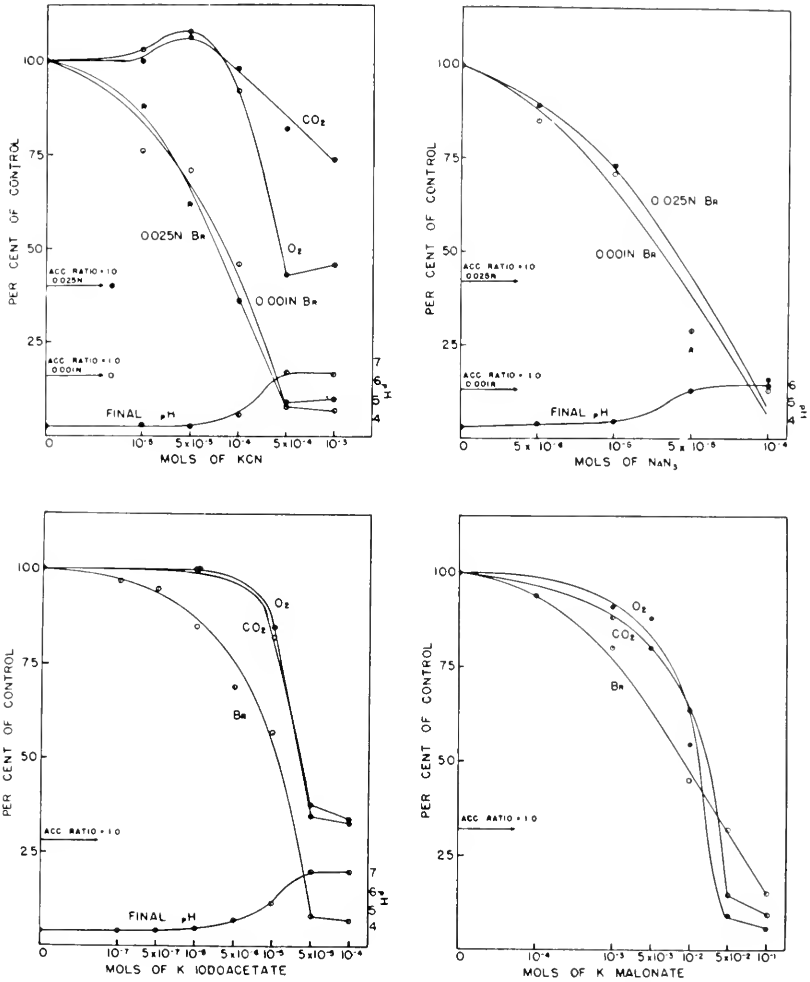

FIGURE 22. Effects of inhibitors on respiration rate and bromide absorption by abscised barley roots. *Upper left:* The effect of cyanide on bromide absorption from KBr solutions initially $0.001\,N$ and $0.025\,N$ with respect to Br and on the gas exchange from solutions initially $0.005\,N$. *Upper right:* The effect of azide on bromide absorption from KBr solutions initially $0.001\,N$ and $0.025\,N$. *Lower left and lower right:* The effect of K-iodoacetate and K-malonate, respectively, on bromide absorption and the gas exchange from KBr solutions initially $0.005\,N$. All solutions also contained $0.001\,N$ KH_2PO_4 and had initial pH values of 4.7–5.7. The points on the ordinates labeled "acc. ratio" represent internal Br concentrations expressed as a percentage of the control equivalent to the initial external Br concentration.—Machlis *(49)*

against the over-all negative potential of the root surface. The iron, with its variable dual charge in the cytochrome complex, is considered to play a role as a carrier of anions from a higher oxidative level externally to a lower level internally. The cations are visualized as moving through isolated mosaic areas of protoplasm by exchange for other cations, primarily internally produced (e.g., H^+), without the direct expenditure of energy. A different temperature coefficient for the absorption of anions and cations was found by Wanner (*89, 90*) and is used in support of the anion respiration theory. This idea has received some support in a recent discussion by Robertson and Wilkins (*68*). Their diagram of the proposed mechanism is reproduced as Figure 23.

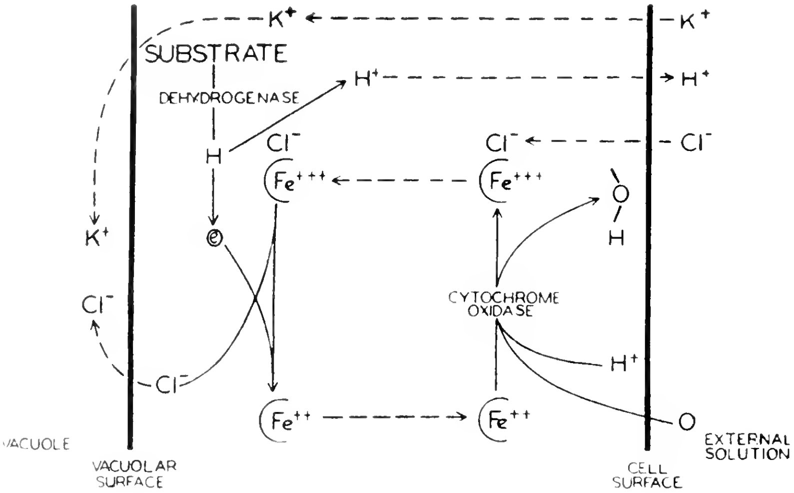

FIGURE 23. Schematic representation of the electron and anion transport system. Solid lines represent chemical reactions; broken lines represent movements of substances; Fe^{+++}, oxidized cytochrome; Fe^{++}, reduced cytochrome.—Robertson and Wilkins *(68)*

The Lundegårdh hypothesis is quite interesting and appears to be in accord with many observations of respiration and accumulation. The limitation which is made between anion respiration and accumulation, rather than inclusion of the necessity for direct expenditure of energy for cation accumulation as well, has been questioned by some investigators. The latter would rather consider oxidative metabolism to be essential to accumulation of both anions and cations. Some theoretical considerations and experimental data still seem to warrant the latter viewpoint.

From a priori considerations, it seems that concomitant expenditure of energy would be required wherever inorganic solute accumulates, irrespective of the mechanism. The test of whether certain solute movement is a spontaneous process or requires work depends on the sign of the energy intensity, or free energy difference, of the substance within the system. The value of such energy intensity differences may be computed from a summation of the number of mole ions of each and all constituents which have moved in a given volume of solution from one concentration (or activity) of the ions to another, again irrespective of mechanism. Such computations include the numbers and concentrations of both cations and anions (*11, 90, 96*). Under some conditions more anions than cations appear to migrate per unit time from external solutions into roots, the excess of the former being partially balanced by cations from other sources (presumably metabolically produced), where inorganic solute is accumulated. Under other circumstances cations appear to migrate at the greater rate or often at nearly equal rates. In any case of solute accumulation, energy is expended—the immediate energy source is usually oxidative metabolism of some sort. The energy requirements for the accumulation of inorganic solute may be estimated from accompanying changes in salt concentrations and may be compared with the concurrent oxygen involved or the carbon dioxide evolved. With certain salts and storage tissues, a close correspondence between the computed energy expenditure and inorganic solute accumulation has been reported (*68*).

Under favorable external conditions of aeration and temperature, little inorganic solute will usually migrate from roots into distilled water (*13*). Two situations may obtain—namely, either a low permeability to such solute (especially at the vacuolar surface) or a dynamic equivalent movement of inorganic solute inward and outward—the latter requiring a continuous expenditure of energy to maintain solute levels. Although a dynamic system is recognized, the importance of a possible low permeability of protoplasm to polar compounds cannot be disregarded. The movement of inorganic solutes is so intimately related to both permeability and energy intensity that the relative importance of the latter two factors is difficult to evaluate (*32*).

The postulate of some sort of inorganic solute-protoplasmic interaction has been accepted by many investigators. Some consider that adsorption may be an initial step in metabolic accumulation. Some time curves of accumulation and the effects of various concentrations of applied salt suggest such a possibility. The shapes of the adsorption exchange curves with time vary with the particular ion concerned. There are, for example, quite different rates of adsorption exchange for rubidium and strontium, for phosphate and iodine, and between these cations and anions (see chapter by Overstreet and Dean). Marked difference also exists between the rates of this process, with a particular ion, for living and for dead tissues. While adsorption is recognized as one mode of salt-protoplasmic interaction, the possibility of specific compounds in protoplasm, characteristic of the species, acting as carriers has been suggested. Such an interaction may be more of the nature of chemical bonding. The salt-protoplasmic interaction may be a bonding of variable strength. Possibly the configuration of the carrier may be labile, the bonding being variably strong or weak and intimately related to metabolism. Perhaps this transition may parallel the oxidative level. Thus, a polarized transport of salt in connection with a specific labile complex could be a mechanism for the movement across protoplasmic layers (*1, 21, 35, 92*). Bipolar protoplasmic complexes could account for simultaneous or separate movement of the ions of a salt. Proteins and amino acids have received a prominent place as suggested ampholyte carriers (*7*). Chelation has been considered by some as a possible means of bonding (*8, 35*). The possible role of cytochrome as a carrier has been indicated earlier (*43, 44*).

TRANSLOCATION AND ACCUMULATION

Detailed discussions of the paths and mechanism of transport of inorganic solute and their distribution with time, in plants, can be found in the chapters by Burström and Biddulph. Certain limited aspects of these processes, however, will be presented here.

Early studies with unicellular algae showed the presence of high concentrations of inorganic solutes in vacuoles. Such solute must have passed across the intervening protoplasmic layer from the external medium. Depletion of salts accumulated in the roots is possible through translocation to the shoot. Part of this solute may be transported from vacuoles as well as from protoplasm of a particular cell. Certain experimental observations suggest that the inorganic solute need not move across cells by way of the vacuole. On the contrary, wherever continuity of protoplasm exists—some workers suggest a "symplast" or protoplasmic continuity throughout the plant—inorganic solutes may move along this pathway as well (*3, 11*).

Numerous interrelations, relative to inorganic solute absorption and translocation, exist between the roots and shoots of plants. A suggestion has been made that light may indirectly control the absorption and retention of inorganic solute by the roots of plants. Lepeschkin (*41*) has proposed a contrasting formation and destruction of compounds within shoots, in dark and light, respectively, which may alter the differential permeability of roots. The loss of inorganic solutes from roots in relation to the illumination of shoots has been reported by others (*1, 48*). That accumulation and retention of inorganic solute may be indirectly related to illumination was recognized early. In these latter experiments with algal cells, the effect was considered to be concerned directly with carbohydrate supply, which, as has been pointed out earlier, may control the rate of accumulation under certain circumstances. Although the carbohydrate factor is recognized, some still consider a possible secondary effect of illumination on salt level in roots.

There are pronounced effects of various substances on growth and metabolism. The levels of auxin, produced in shoots and transported to roots, can modify conditions and processes in the latter organ. Although a direct effect of auxin on inorganic solute accumulation by roots has not been clearly shown (*47*), modification in the rates of exudation and the inorganic solute concentrations therein imply some indirect effect on salt migration (*73*). This may be through permeability phenomena, but further evidence is necessary to this point. Possibly data are even now available to clarify these effects and they may be elucidated in another monograph on hormonal phenomena.

A number of experiments indicate a close interrelation between root and shoot relative to oxygen supply. The effect of partial pressure of oxygen in the bathing medium of abscised roots on inorganic solute accumulation has been pointed out earlier. Studies with intact plants suggest that some species, for example rice, might develop and carry on internal processes at lower oxygen levels in the bathing medium than others. The similar aerobic requirements for accumulation of inorganic solute by abscised roots of rice, barley, and tomato were clearly shown in Figures 8A and 8B. Earlier observations indicating decreased growth of some species under adversely low oxygen conditions in soils were confirmed by Vlamis (*88*). Plant development under good and poor drainage is indicated by the fresh weights of plants in Table IX. Potassium analyses on roots of these plants indicate no adverse effect of treatment on accumulation with rice, but a reduction in the same process with barley and tomato, particularly the latter. Controlled experiments with solution cultures supplied with various

TABLE IX

Fresh Weight and Potassium Concentration of Expressed Sap of Plants Grown 6 Weeks in Drained and Submerged Clay.—Vlamis and Davis (*88*)

Plant	Soil Treatment*	Shoots		Roots	
		Fresh weight	K conc.	Fresh weight	K conc.
		g.	m.e./l.	g.	m.e./l.
Barley	drained	18.0	167	2.2	57
	submerged	2.0	—	0.2	—
Tomato	drained	12.2	—	1.2	—
	submerged	2.7	—	1.4	—
Lowland rice	drained	20.0	196	9.2	55
	submerged	34.5	209	38.6	32

*Clay soil

Note: Submerged plant roots were gradually exposed to increasing soil moisture until the water level was one inch above the soil surface. Plants of both sets were frozen and sap expressed and analyzed for potassium concentration.

levels of oxygen were made. The growth of plants and potassium accumulation by roots of three species are reported in Table X (*88*). This shows the variable effect for these species, of oxygen supply to roots from its bathing medium. The inference, which is borne out by evidence of others (*14, 95*), is that the requirements for oxygen in some species is met by a transport of this gas from the shoots to the roots. This implies a difference in anatomy, or some oxygen transport process within the various types of plants. Anatomical studies showing the development of air passages favor the former assumption (*21*). The data of Vlamis suggest a most favorable position for rice, an unfavorable position for tomato, and an intermediate position for barley, in this regard. His data, in accord with others referred to earlier, show the very harmful indirect and direct effects of relatively high concentrations of carbon dioxide in the environment of roots.

TABLE X

Fresh Weight and K Concentration of Sap of Plants Grown outdoors 6 Weeks in Culture Solution with Gas Exposure.—Vlamis and Davis (*88*)

Plant	Root Treatment	Shoots	Roots	
		Fresh weight	Fresh weight	K conc.
		g.	g.	m.e./l.
Barley	control	49.2	22.6	103
	air	53.5	25.3	98
	methane	20.2	10.2	58
Tomato	control	41.8	17.4	102
	air	64.5	31.0	101
	methane	3.2	1.2	—
Lowland rice	control	25.5	11.1	100
	air	25.0	15.3	101
	methane	24.0	24.7	97

NOTE: Methane gas contained less than 0.02 per cent of oxygen.

Although transpiration is considered unnecessary and certainly not directly essential to accumulation of inorganic solute by the roots of plants, an indirect influence on continued absorption may obtain under certain circumstances. The inhibiting effect of a high-salt condition in roots on further accumulation was pointed out earlier. Transport from root to shoot may lead to a more rapid, continued salt absorption by roots in such a condition. The indirect influence of transpiration in enhancing the rate of translocation in some cases is clear, though probably unimportant under usual growth conditions (*12*).

DISTRIBUTION PATTERNS

The distribution pattern of inorganic solute within plants is related to the relative rates of accumulation by cells, translocation, and growth. This subject will no doubt be presented by Biddulph elsewhere. It may be well, however, to discuss certain observations in this direction. Accumulation of salt in cells, it has been pointed out, is intimately connected with permeability and the process of metabolism. The energy supply for metabolic or active accumulation has its source in protoplasm. It is of interest to see, if possible, whether a process of secretion of inorganic solute from protoplasm to vacuoles or to stelar vessels is concerned (*2, 3;* compare *46*). Using unicellular algae for material, Brooks has shown that accumulation levels of ions are, at least initially, higher in the cytoplasmic fraction (*9*). Some experiments have indicated a higher concentration of certain ions in the vacuolar fluid than that in the residual portion of the cell samples (*32*). Such results are presented in Table XI. From these data it might be inferred that a secretory process exists. However, this observed difference between lumen and protoplasm is probably related to less obvious moisture relations within the protoplasmic fraction (*11*). Thus, for example, a high nonsolvent water percentage under natural conditions in protoplasm might decrease the usual solute concentration through dilution, on expression of composite sap from this fraction. In such a way, the observed concentration differences between vacuole and aqueous solution phases of cytoplasm might be of opposite sign from those obtaining under natural situations.

Across the root, the transverse pattern of accumulation level is less known. Some data, including exudates from decapitated plants, may indicate a secretion from living cells to stelar lumina (*32*). However,

TABLE XI

Absorption of Rb* and Br* Ions by Nitella

Experimental Conditions	Rb* or Br* in Counts per Min. per Ml.			Ratios of Rb* or Br* in Counts per Min. per Ml.			Spec. Cond. in Mhos 10^{-3}	
	Sap		External solution	Vacuole / Residue	Vacuole / External solution	Residue / External solution	Vacuolar sap	External solution
	Vacuole	Residue						
Experiment 1								
Rb*Cl aerated, light 1 day	82	417	33	0.2	2.5	12.6		
Rb*Cl aerated, light 10 days	400	545	33	0.7	12.1	16.5		
Experiment 2								
Control cells (initial status)							6.9	
KBr* aerated, light 23 hr.	5180	3840	280	1.4	19	14	8.9	0.2
KBr* aerated, light 71 hr.	12000	6750	280	1.8	43	24	10.4	0.2
Experiment 3								
Rb*Cl aerated, light	74	—	30	—	2.5	—		
Rb*Cl, N gas, dark	23	—	24	—	0.7	—		

NOTE: Experiment 1: *Nitella* cells from pond water. Nodal cells and terminal buds removed from cells of main axis of thallus; only these latter cells were used in absorption studies. The experimental external solution was Rb*Cl, 1.0 milliequivalent per liter.

Experiment 2: *Nitella* cells from pond water. Nodal cells and terminal buds removed from cells of main axis of thallus; only these latter cells were used in absorption studies. The experimental external solution was KBr*, 1.18 milliequivalents per liter.

Experiment 3: *Nitella* cells from pond water. A composite group of cells was used during the absorption period. Vacuolar sap was obtained from large cells of the composite sample. The experimental external solution was Rb*Cl, 5 milliequivalents per liter.

similar reasoning to that suggested for distribution in single cells may be invoked. Other evidence on the transverse pattern of salt accumulation among the various cells of the root is wanting. Anatomical considerations, along with postulated oxidative levels, suggest accumulation gradients of decreasing concentration progressively inward from the external surface. Since all cells of the cortex may be bathed by fluid similar to the external medium of roots (*62*), the radial gradient across this tissue may be small. Consideration of a controlling influence by the endodermis on rate of translocation and oxidative levels of the

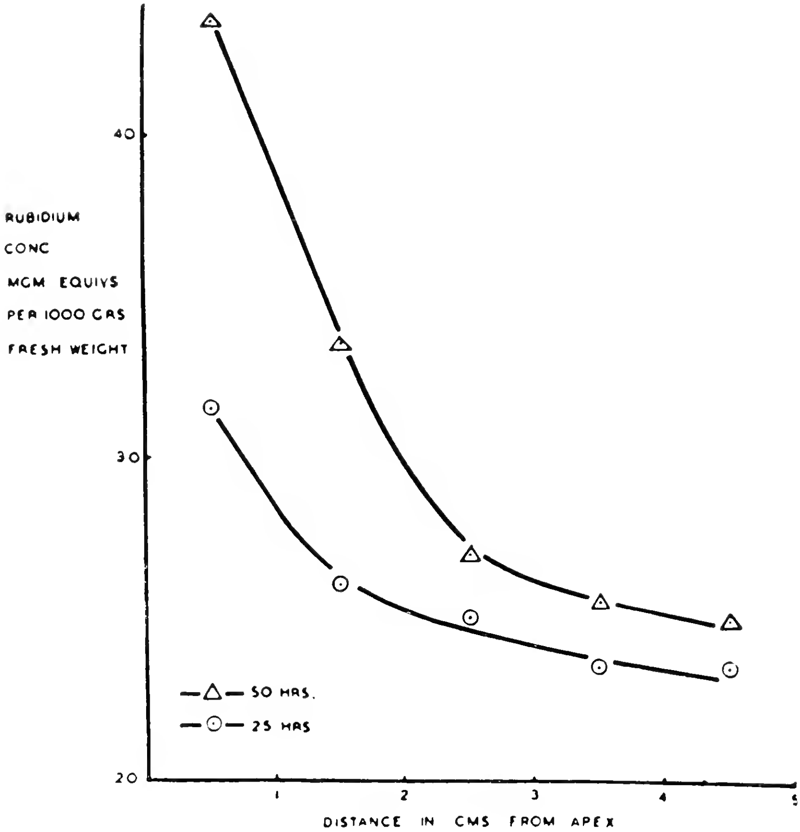

FIGURE 24. Distribution of rubidum in excised barley roots showing the effect of time. Culture medium 0.005*M* RbBr.—Steward et al. *(79)*

distinctly different types of tissue external and internal thereto may not be without merit (*21*). Conditions within the cortex and its relative volume certainly favor high levels of accumulation as well as a large quantitative proportion of inorganic solute therein (*61*).

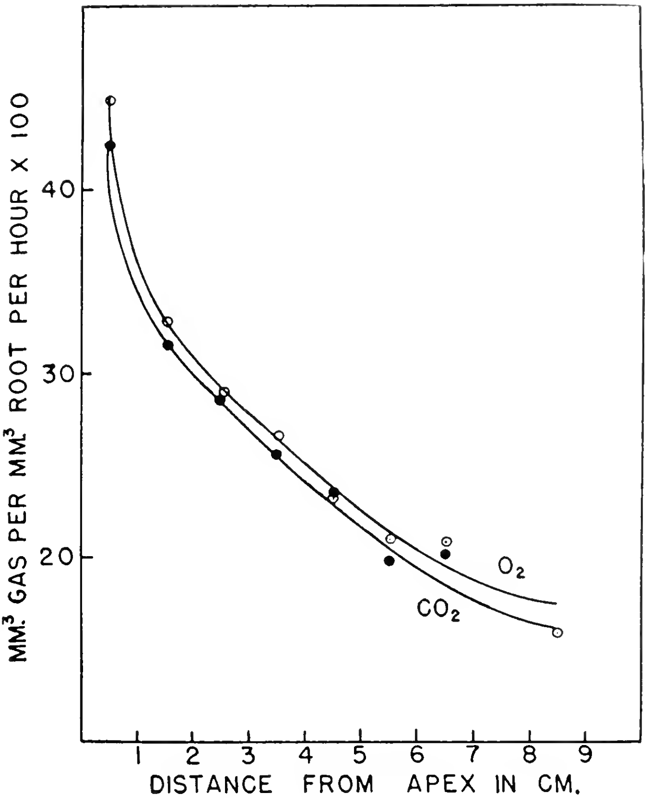

FIGURE 25. The gas exchange of successive segments of excised barley roots. Measurements made on 72 segments in 2 ml. of dilute nutrient solution; temperature 24° C.; experimental time, 2 hours.—Machlis *(50)*

The longitudinal pattern of inorganic solute distribution in roots has been observed by several investigators. One of the first experiments, under controlled conditions of short time study, was that by Prevot and Steward (*61*) using abscised roots. They showed that inorganic solute

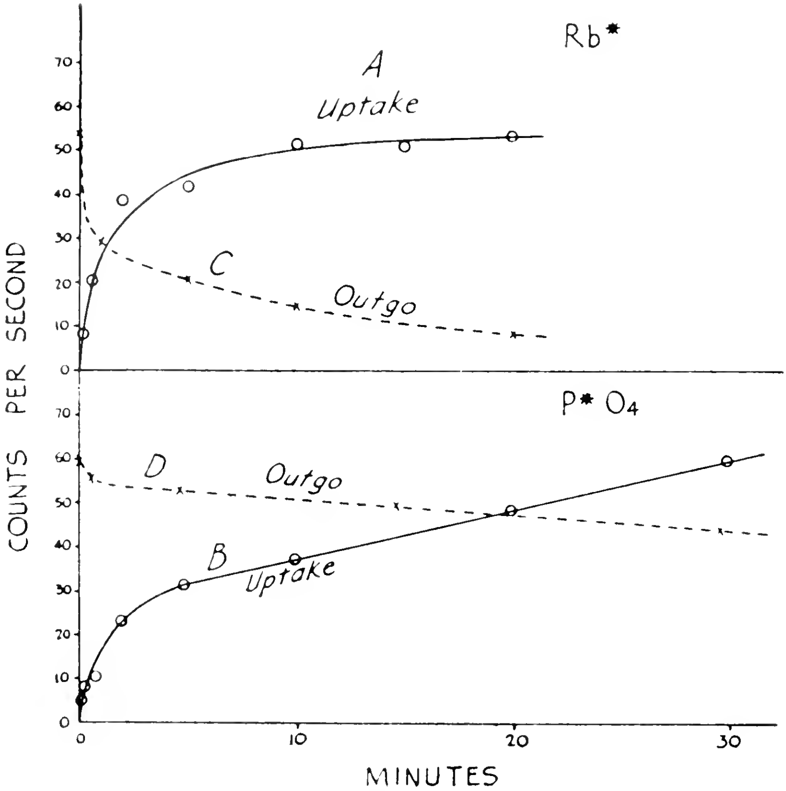

FIGURE 26A. Graphs here and in Figure 26B show the absorption at 0° C. of Rb^{86}, P^{32}, Sr^{85}, and I^{131} by apical root segments and the subsequent release of the elements in exchange for inert isotopes. In 26A root segments 2 cm. in length and about 0.4 mm. in diameter were repeatedly immersed in Rb*Cl or KH$_2$P*O$_4$; in 26B, in Sr*Cl$_2$ or KI* solutions. Following each immersion the segments were washed in distilled water and counted (curves A and B). The segments were then transferred to 0.005N nonradioactive solution of the same salts and the process of successive immersions repeated (curves C and D). The amount of radioelement in each segment is expressed in terms of counts per second.—Overstreet and Jacobson *(56)*

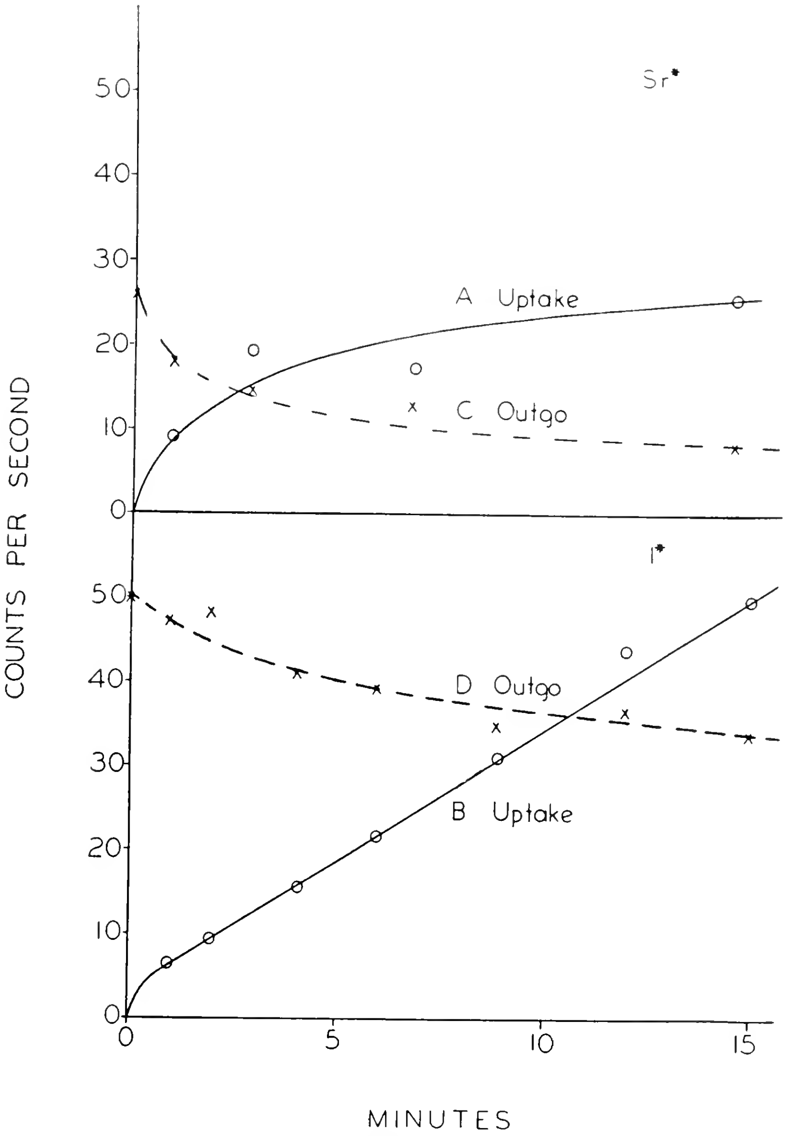

FIGURE 26B. These graphs are discussed under Figure 26A.

may be accumulated throughout the length of unbranched roots of succulent species. Concentrations of bromide at various levels were represented in Figure 4. At the time of measurement, a gradient of concentration was evident, decreasing from the meristematic distal end toward the proximal or root-stem transition part of the root. A similar distribution was shown spectrographically for potassium and rubidium by Steward, Prevot, and Harrison (79). The similarity in shape between a distribution curve for metabolically accumulated rubidium (Figure 24, from 79) and a respiration gradient curve (Figure 25, from 50) on the same types of roots is probably further evidence for the

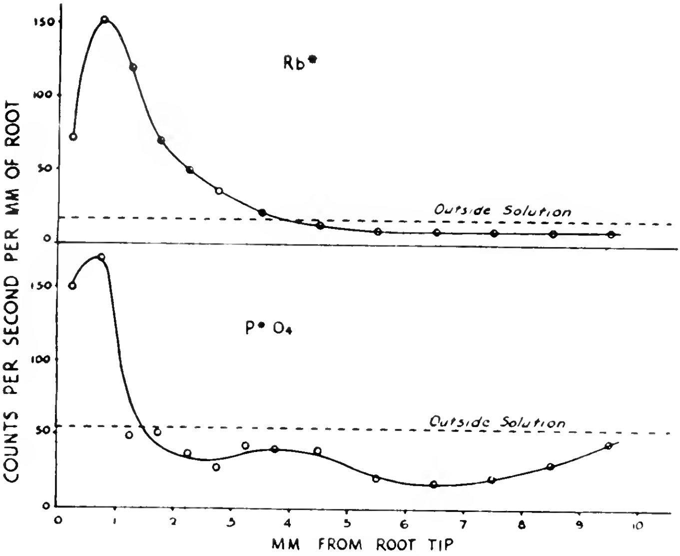

FIGURE 27A. Graphs here and in Figure 27B show the magnitude of nonmetabolic absorption of Rb^{86}, P^{32}, Sr^{85}, and I^{131} (expressed in counts per second per mm.) as a function of distance from root apex. In 27A, apical segments of barley roots 1 cm. long and approximately 0.4 mm. in diameter were immersed in Rb*Cl or $KH_2P^*O_4$; in 27B, in Sr^*Cl_2 or KI* solutions for 30 minutes at 0° C., and subsequently sectioned and counted. The dotted line labeled "outside solution" in each case corresponds to the activity of a volume of the bathing solution equal to that of 1 mm. of root segment.—Overstreet and Jacobson *(56)* close relationship between these two processes (see also 25). The shape of the distribution curve for adsorption exchange along the same types of barley roots may be slightly different. Curves of this sort are represented in Figures 26A, 26B, 27A, and 27B, from Overstreet and Jacobson (*35, 56*). These show the differential adsorption pattern of various anions and cations in short time experimental treatments.

The salt distribution pattern in the plant as a whole will depend on many interrelated factors including relationships between shoot and root, organ and organ, tissue and tissue, and among cells of like type; for the plant is an integrated machine, the process in any one part being dependent upon the concurrent and past action and conditions in every

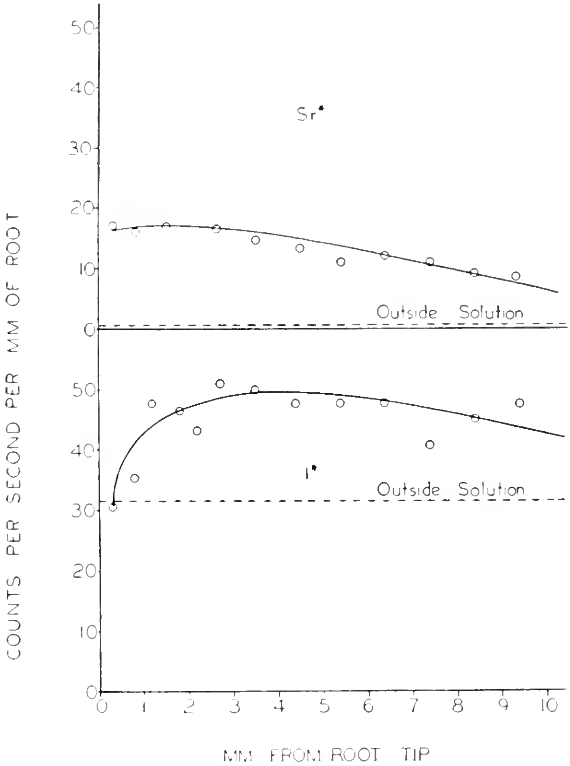

FIGURE 27B. These graphs are discussed under Figure 27A.—Jacobson and Overstreet (*35*) other part. The concentration pattern for inorganic solutes generally is in accord with the idea of a relationship between rates of accumulation and metabolism. It should be sufficient to indicate here that metabolic accumulations usually occur in growing parts of shoots, in young leaves, in immature fruits, and seeds.

SUMMARY

Inorganic solute accumulation in roots is a process characteristic of plant cells which are situated in favorable environments. Like all physiological processes, the rates of accumulation are altered by external and internal conditions and the concurrent rates of certain related processes. The qualitative and quantitative limits for any particular process (here, especially inorganic solute accumulation) are controlled by the genetic potentialities of the specific physico-chemical species.

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CHAPTER

9

The Mechanism of Ion Absorption

HANS BURSTRÖM

During recent years there have appeared complete theories of the mechanism of the active absorption of ions, which in a rather definite way have brought together the vast experiences connected with the subject. It is an easy task to explain an active absorption and accumulation of one kind of ion, and this can be brought about in experiments with models of different kinds. However, the crucial point in every such theory is to explain the simultaneous absorption of anions and cations and their accumulation together against the diffusion gradient.

The first of these theories was advanced by Lundegårdh (*3*), and was founded on experiments in progress since 1933; a second theory, a modification and development of the former, has been advanced by Robertson (*6*). Although the main features of both theories are probably well known to you, I feel it necessary to review them in some detail before entering into a discussion of the present situation of this field of research.

Lundegårdh's theory is based on four assumptions, which perhaps today may be regarded as generally accepted facts. 1) The absorptions of anions and cations are independent of each other to such an extent that different mechanisms must be responsible for each. 2) The absorption of cations takes place in two steps, one involving the absorption of ions from the external solution and the other the excretion of the ions from the cytoplasm into the vacuole. The latter step constitutes the real accumulation. A number of recent investigations have corroborated this view for different plant materials such as roots, storage tissues, and even leaves. Arisz (*1*) has shown that leaves of *Vallisneria* carry out active accumulation of ions in essentially the same manner as other organs. It has also been verified by several investigators in experiments with tagged cations that the first step of the absorption is reversible and is probably, as was assumed long ago, an adsorption or a tendency toward a Donnan equilibrium on the surface of the plasma colloids. 3) The absorption of anions is of a different nature. It is irreversible and, as has been shown with tagged ions, it takes place not only against the diffusion gradient but also against the charge of the cell (which is predominantly negative) and the adsorption potential. It depends on a portion of the total respiration which is usually called anion respiration and which Robertson has called salt respiration. 4) This anion respiration is different from the rest of the respiration which is called the ground respiration.

Some of these points, especially point three, the connection of accumulation of anions with respiration, have been questioned, but a number of new data, particularly from Robertson, support the assumptions made by Lundegårdh.

New evidence in favor of the opinion that respiration is connected specifically with the intake of anions is found in the determinations of the effect of temperature on ion absorption. I may cite the experiments by Wanner (8). These and other investigations without exception show a significantly higher, sometimes very much higher, temperature coefficient for the absorption of anions than for the absorption of cations. Wanner found a Q_{10} of 1.4 for the absorption of potassium from potassium nitrate but a Q_{10} of about 2 or 2.5 for the absorption of the nitrate ion. With ammonium chloride the corresponding figures were 0.9 for the cation and 1.5 for the anion. Still greater differences have been recorded by Jacobson and Overstreet. A low temperature coefficient is characteristic of physical reactions, whereas chemical transformations normally have quotients of two or three. These observations, therefore, support the view that the first step in the absorption is a physical phenomenon, while the second step is connected with metabolism, and that this applies especially to the absorption of anions.

Robertson and his collaborators further have verified the point that respiration is directly connected with the process of ion absorption. Not only does the total respiratory rate increase to a higher level if salt is added to the external solution, as demonstrated by Lundegårdh long ago, but also, if salt is withdrawn from the external medium, the rate again goes down to approximately the initial level. Thus, this effect of salt on the respiration is reversible, which it would have to be, if it is connected with the absorption of ions and not only with the presence of ions in high concentrations within the tissues.

This salt respiration has been further defined by its high sensitivity to the heavy metal inhibitors—hydrocyanic acid, carbon monoxide, and sodium azide—to which agents the ground respiration is almost insensitive. This point, initially brought out by Lundegårdh, has been verified by several investigators who have shown that the intake of ions can be wholly inhibited by hydrocyanic acid. Especially convincing are the experiments of Robertson, according to which an addition of hydrocyanic acid stops both salt intake and the associated increase in rate of respiration at once, but leaves the ground respiration almost unaffected. Consequently, with roots or tissues in water, hydrocyanic acid has little effect on the respiration.

According to prevailing concepts, this would indicate that the salt respiration is catalyzed by hemin compounds, while the ground respiration is catalyzed by a different enzyme system, the nature of which is unimportant in this connection. The hemin compounds contain iron as the active constituent with which the mentioned inhibitors form complexes and thus inactivate the enzyme.

On the basis of the above evidence, Lundegårdh assumed, as did Robertson later, that the salt respiration is catalyzed by cytochrome systems, because cytochromes are probably universally present and contain hemin compounds which are easily inactivated by hydrocyanic acid. Lundegårdh and Robertson go even further and claim that the cytochromes themselves play a fundamental role in the absorption of the anions. This remains to be proved, but is at present an integral part of the theories advanced by both men.

The active part of the cytochromes is the iron atoms which change their valence by taking up and giving off electrons according to the formula, $Fe^{+++} + e^{-} \rightleftarrows Fe^{++}$. Thus the cytochromes serve as carriers of electrons and function at one end of the respiratory system. Now it is assumed, or is really an established fact, that when the Fe^{+++}

ion is formed it can attract one additional negatively charged particle; that may be an electron as in the above case, or it may be an anion equivalent (A^-). The A^- may denote any mineral anion. As soon as ferrous iron is oxidized by giving off an electron and becomes trivalent, it can bind an A^-. That implies that there may be an exchange on the iron atoms of electrons for anions. The iron loses one electron and attracts one anion.

To explain the transport of the anions through the cytoplasm, Lundegårdh assumes that there are tracks or bridges of cytochromes arranged in transverse direction across the cytoplasm between its outer boundary, called the *o*-level, and the inner boundary, the *i*-level, which may be the tonoplast or central parts of the organ. Along these tracks the anions are moved by successive exchanges for electrons. At the *o*-level the anion is attracted by a ferri-cytochrome. This then takes up an e^- from an inner cytochrome, loses its anion, which is caught by the second cytochrome, and so on. In this way the anion moves from one iron atom to another through the system.

Lundegårdh suggests that there are waves of electrons going from the *i*- to the *o*-level and, if such a transport of electrons occurs, anions can be moved in the opposite direction. At the *i*-level the anions are released from the cytochromes and given off to the vacuole. It is of course impossible, for electrostatic reasons, for a single anion to be released unless it be combined with a partner having the opposite charge, a cation. The anions are thus combined with cations at the *i*-level. The cations may be hydrogen ions or more likely metal ions, which are abundantly present, and thus the salt becomes stored in the vacuole.

The cations can easily be bound to the cytoplasm, because we know, especially from Lundegårdh's determinations of the surface potentials of the cytoplasm, that it has a predominantly acid character. It contains abundant acid groups with dissociating hydrogen ions, and at these loci, metal cations can be adsorbed by an exchange for the hydrogen ions. I have already mentioned that there are good reasons to assume that in the first step of the absorption the cations really are attached to the plasma by such an adsorption. Lundegårdh now assumes that the cations are transported through the cytoplasm by repeated such exchanges for hydrogen ions and ultimately reach the *i*-level. Repeated exchanges of this kind must take place to secure the adsorption equilibria as the cations are withdrawn from the colloidal system through combination with the released anions at the *i*-level. Thus, the driving force for the intake and accumulation of salts is the absorption of anions which is caused by the passage of electrons to the plasma surfaces.

The central problem then becomes to ascertain whether we are correct in assuming such a unidirectional wave of electrons. Lundegårdh answers this question in the affirmative. The transport of electrons by cytochromes is a well-known part of the iron-catalyzed respiratory system, one end of which is the oxidation by the atmospheric oxygen of ferrous iron carrying one electron in excess. In fact, the oxidation requires that the ferrous iron give off its electron to the oxygen, which combines with hydrogen ions to form water. This is the formation of water which occurs in the respiration. This oxidation, Lundegårdh postulates, ought to take place at the *o*-level, because oxygen is supplied from the external solution, and there must be a falling oxygen gradient from the *o*- to the *i*-level. The other end of the respiration, involving dehydrogenations of the respired substrate, is a reduction process and may be restricted to loci of low oxygen tension, which means the *i*-level. Electrons must be given off there and carried through the cytoplasm to the *o*-level. We get as a consequence the postulated transfer of electrons, and thus the whole mechanism is made possible. The production of electrons is caused by the splitting of the substrate as a normal part of respiration which together with the oxidation at the *o*-level appears as the salt respiration. In this way it is clear that the absorption of anions is directly connected with the process of respiration.

Such a picture involves a fixed polar organization of the cytoplasm in accordance with the oxygen gradient, for which we have no direct evidence. It is absolutely necessary, however, for any theory of ion absorption to assume a polarity within the cell, and this one seems to be both very simple and well founded. The ultimate controlling factor of the absorption and accumulation of ions is then the oxygen gradient in the cell.

The theory of Robertson is in its main features very similar to that of Lundegårdh, on which it is largely based. Robertson assumes the same function of the cytochromes, the same polarity, and the same mechanism of combination of anions and cations at the *i*-level. He has made one change, however, which may be rather important.

He does not postulate a successive exchange of anions along a cytochrome bridge and against an electron wave, but assumes that the cytochrome system circulates with the cytoplasm between *o*- and *i*-levels. At the *o*-level, where ferric iron is formed by oxidation, it catches an anion and the whole complex is moved to the *i*-level. Here the iron is again reduced by absorbing an electron and the anion is set free. The iron, in the reduced state, returns to the *o*-level, where it is oxidized, thus completing the cycle. It is worth mentioning that Arisz (*1*) has pointed out a third possibility, namely, that the transfer of ions between the plasma constituents is conditioned by the continuous breaking down synthesis and of organic compounds, and this means that a high degree of instability of the cytoplasm should be one of the causes of the change in position of the mineral ions.

At present we cannot decide which of these two or three assumptions is correct, but this is certainly an important question, because it may contribute to an explanation of the quantitative relationship between the salt respiration and the salt accumulation, or, which is the same thing, the efficiency of the salt respiration. It is quite certain that there is no stoichiometric relationship between salt respiration and the amounts of ions absorbed. Robertson has computed, however, the maximum amount of ions that can be absorbed per unit of respiration if the theory is correct. This is very simple since one electron corresponds to one equivalent of anions and one molecule of oxygen corresponds to four electrons. Thus, one molecule of oxygen consumed in respiration may cause the absorption of, at most, four equivalents of anions. His experimental data show that the highest ratios of ion equivalents accumulated to oxygen consumed for storage tissues and excised roots are between three and four. This is no proof of the correctness of the theory, but is quite consistent with it. In a very recent paper Lundegårdh (5) has shown, however, that in intact roots this quotient does not exceed 1, which is explained by a respiration connected with an internal transport of salt in the normally functioning roots. In practice, it is not the same thing to work with a histologically highly organized root as with a uniform piece of storage parenchyma.

So much for the theories. They are obviously speculations to some degree, but, nevertheless, in conformity with all or most experimental data and also with our knowledge of respiratory mechanisms. Several points need to be further clarified, however.

The first question is, as already discussed by Robertson, why this special respiration system does not work in the absence of external salts. All integral parts are present in the cell: substrate, enzyme, and oxygen. Here we can only guess. We may refer to an activation of the system by the anions, but that does not explain anything. A second point is, how is it possible to get unequal absorption of anions and cations? If the anions are absorbed in excess, the problem is simple. The excess is combined with hydrogen ions and the vacuolar sap is acidified. The cell possesses no means of preventing such a decrease in pH. Usually there is, however, an excess accumulation of cations, because the rapidly absorbed anions, notably nitrate, are assimilated to a large extent in the cytoplasm and an excess of cations appears in the vacuole.

These two questions are probably intimately connected. The second one has been partly elucidated by the work of Ulrich (7) and the author (2). Ulrich showed that the excess of cations in his material was balanced by a production of malic acid which was equivalent to the excess cations, with some exceptions. Our observations showed that this equilibration without exception takes place not at the point of intake of the ions but at the point of their accumulation or, with the adopted terminology, at the *i*-level. When an excess of cations is released to the vacuole, it is accompanied by an equivalent amount of malic acid or, at the prevailing pH, of malate ions.

As to the source of this malic acid, it is almost certainly formed as an intermediate in the acid cycle of the respiration. This acid cycle forms the fundamental part of the dehydrogenase systems and is, according to the picture outlined above, localized to the *i*-level. In this cycle malic acid may be formed from succinic acid and itself transformed into oxaloacetic acid. Of course other transformations are also possible. Here malic acid, which is a weak acid, reacts as the undissociated acid, but it must always be dissociated to a fixed extent according to the prevailing pH, malic acid $\rightleftarrows$ malate^{--}. If an excess of cations is added to such a system, this dissociation shifts to the right, and an amount of malate equivalent to the cation excess is withdrawn from the respiration cycle and is apparently excreted together with the cations into the vacuole. If the excess diminishes, the dissociation reaction is reversed and the acid disappears in the respiration cycle again. This is a simple matter of equilibria, and Ulrich has shown directly that the formation and disappearance of the malic acid in connection with the ion accumulation is effectuated by the respiration system.

We may go one step further. If the ferric ions of the cytochromes are able to bind anions, why should they restrict themselves to the mineral anions, why not attract organic anions as well? Robertson has, in fact, raised this question, and Lundegårdh (*4*) has answered it by assuming that in intact roots, a major part of the salt respiration depends upon the action of a respiration system combined with organic anions, called "native" anions. If we assume that the ferric atoms are normally equilibrated by malate, or other organic acids formed in the cell, it is easy to explain why an excess of cation accumulation causes malate formation.

As soon as a mineral anion enters the cytoplasm, it is attracted by a ferri-cytochrome and there replaces a malate ion, which disappears in the respiration. Then the anion is moved inward and, if it should happen to be assimilated as regularly occurs with nitrate ions, its place is immediately occupied by a malate ion again. At the *i*-level the anions (mineral ions or malate ions) are set free and combined with cations in the vacuole (Figure 1). It is, of course, not necessary to assume that this role must be played by malate or malic acid, although it is so in the cases investigated so far. In other plants other acids may fulfill the same function, depending on, for instance, the existing pH of the cytoplasm, the dissociation constants of the acids, etc.

A very strong argument in favor of the assumption that the dehydrogenation system, producing both hydrogen and organic acids, is located at the *i*-level where the accumulation of the ions is regulated, is given by Lundegårdh (*4*) in a very recent paper. He has shown that the bleeding from a root is dependent upon the release of ions to the vessels, which is a reaction at the *i*-level, an anaerobic reaction. This is inhibited by such agencies as iodoacetate and fluoride, known to attack the dehydrogenase systems or the anaerobic process of glycolysis.

If it is true that the cytochrome system may be occupied by organic acids, it follows as a consequence that the salt respiration system might work with the organic acids present in the cell and not only when mineral salts are added externally. That is just what Lundegårdh has assumed to occur, and it is supported by other indications in the same direction. For instance, Robertson has shown that a respiration sensitive to hydrocyanic acid also occurs in the absence of mineral salts under certain conditions. Inhibition by hydrocyanic acid may cut off not only the whole additional salt respiration but a part of the apparent ground respiration as well. These and other observations show that the cytochrome-regulated respiration is not exclusively dependent on the addition of salt. It is apparently only a question of relative rates of respiration with and without mineral salts. Salts, presumably anions, greatly activate a respiratory system, and it may be assumed that this activation involves the exchange of the organic acids for the mineral

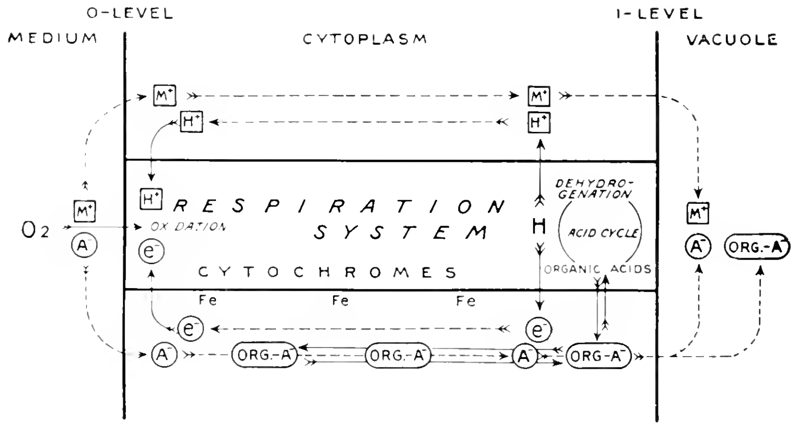

FIGURE 1. Scheme for the active absorption of ions. The salt respiration system is oriented transversely across the cytoplasm. At the inner, dehydrogenation level, protons and electrons are produced, and they are moved to the outer, oxidation level in exchange for salt cations and anions, respectively. Solid arrows denote only chemical reactions or equilibria; broken arrows, mechanical transport.

anions. This means that the cytochrome system saturated with organic anions should be much less active than when it contains attached inorganic anions. The function of the cytochrome system depends upon its ability to carry electrons between the iron atoms. If the latter bind anions, the activity must depend upon the rate of exchange of the electrons for the anions, a reaction which certainly must vary with the kind of ion. The fact that large bivalent organic anions are much less exchangeable than the inorganic ions, might explain the activation of the respiration system by the added mineral salts.

In this way the organic acids occupy a central position of interest. Their role is, however, far from clarified and can hardly be tackled from common physiological points of view. If this theory gives a true picture of the mechanism of the ion absorption, biochemical studies are necessary for further progress within this field.

There are still other difficult questions to answer. One is the real cause of the specificity of the ion absorption: why do not all plants absorb all ions in the same proportions, for instance always potassium more rapidly than sodium, as was to be expected from the physical properties of the elements and their importance for the exchange phenomena regulating the absorption. It is obvious that the recorded theories can only claim to explain the principles and the fundamental mechanism underlying the absorption of ions, but there are details which hitherto have not been seriously considered at all. I do not think, however, that such gaps in the theories detract from their value as a solid working basis for explaining the active absorption of ions.

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CHAPTER

10 The Translocation of Minerals in Plants*

O. BIDDULPH

In multicellular organisms where one organ specializes in mineral absorption, a distribution system for those minerals is an inevitable development. The efficiency of the distribution system will, in general, reflect the over-all efficiency of the organism since the growth rate is dependent on an equalization of the necessities for growth between the various organs and tissues. The growth rate may not outstrip the supply of material upon which it is dependent.

In plants there is no concentrated expenditure of energy for either maintenance of body temperature or for muscular contractions, hence, in keeping with the general level of specialization, there is no need for a highly developed "circulatory system." Nevertheless, the translocation system must be efficient enough to distribute rapidly the incoming materials, i.e., minerals, and to equalize concentrations between various parts as tissue elaborations proceed. Even under the conditions of reduced growth rates there is a continual exchange of materials within the physical structure of the protoplasm which demands a constant supply and removal of the basic components. The cellular entities, such as the major morphological protoplasmic constituents, maintain a relatively constant pattern even though the molecular or atomic constituents undergo continual replacement. It is my purpose here to review the translocation of minerals in plants with a view to correlating some of the observations on translocation with the requirements of the organism. It will be necessary in many instances to resort to

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*The writer gratefully acknowledges the active participation of J. Witt, J. Rediske, and C. Woodbridge in the research upon which parts of this report are based. Figures 1, 5 and 8, and 4 and 7 are from their respective theses in the Department of Botany at the State College of Washington.

generalizations. I realize that generalizations can be dangerous, yet it is through them that progress is made. For the purpose at hand, I shall attempt to travel that narrow path between oversimplification on the one hand and undersimplification by too ardent a treatment of minutiae on the other.

TRANSLOCATION ACCOMPANYING GERMINATION

The translocation of minerals from the storage regions of the seed to the developing plumule, hypocotyl, and radicle constitutes the initial mineral translocation in the ontogeny of the developing plant. Accompanying the hydration of the seed there is a solution of the minerals present. Most seeds contain sufficient minerals to serve the developing seedling until the roots, stems, and leaves are well developed.

During the germination of red kidney bean seeds in moist Scot towels, we have observed a net loss of iron from the seeds amounting to approximately 50 per cent of the total initially present. This amount of iron was gained by the toweling at the expense of the seeds and, therefore, was lost to the seedlings as they were removed to other media after two days. At this stage the roots were not sufficiently developed to reabsorb the iron from the toweling. The major portion of the iron remaining in the cotyledons was readily translocated to the developing plumule, hypocotyl, and radicle. This translocation parallels closely the translocation of carbohydrate (Figure 1).

These results are in good agreement with the movement of nitrogen, phosphorus, magnesium, and potassium in germinating wheat seeds as reported by LeClerc and Breazeale (5). This is true even though the storage materials in wheat are found chiefly in the endosperm while in beans the reserves occur in the cotyledons.

Studies were made in both normal daylight and total darkness. Translocation of iron, as well as carbohydrate, proceeds much more slowly in darkness than under conditions of normal daylight (alternating night and day). Both Whitmore (*10*) and Withrow (*11*) have reported somewhat similar results. It is clearly indicated that light exerts an influence on the rate of translocation of both reserve foods and iron from the cotyledons into the developing plant axis. The mechanics of this effect is unknown at present.

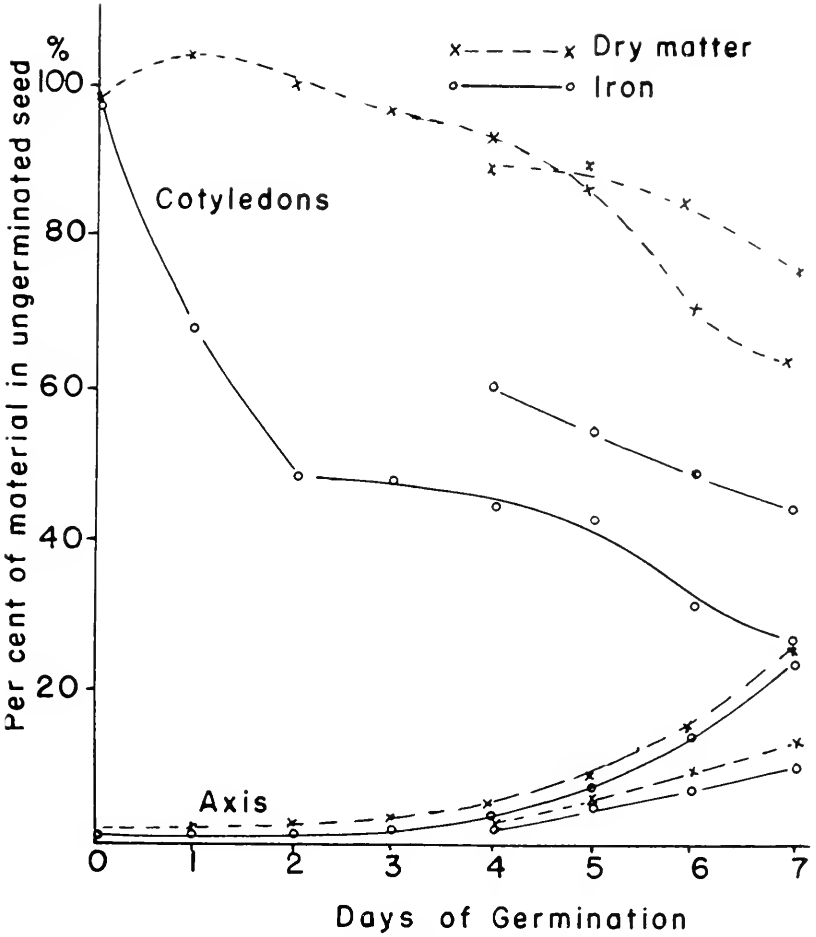

FIGURE 1. The dry matter and iron content of bean seedlings during the first seven days of germination expressed as percentage of material in ungerminated seeds (less seedcoat). Graphs for plants kept in total darkness are included for the fourth to the seventh day only.

TRANSLOCATION OF MINERALS IN THE XYLEM

Translocation in the mature plant is a complex phenomenon. It is dependent on the initial absorption processes carried on by the epidermal cells of the root. From this point there begins a cell-to-cell transfer across the cortex to the cells surrounding the xylem. Here, in a process "akin to secretion," according to Hoagland (*4*), the minerals enter the xylem system wherein they ascend to the aerial extremities of the plant. This process of secretion, if it may be so called, is one of the least known of all processes in the realm of mineral translocation. Its elucidation undoubtedly must await a fuller understanding of the absorption process. Since so little is known of this "secretion" process as it applies to translocation, it will not be discussed here.

The delivery of mineral elements to the leaves of mature plants most certainly occurs primarily through the xylem tissues. Stout and Hoagland (*9*) have contributed the most direct evidence bearing on this point. In their experiments the phloem was isolated from the xylem by the insertion of waxed paper between the tissues but the paper left the longitudinal continuity of both elements intact. Radioactive nutrient elements were then placed in the root environment and the path of these elements was followed by the radiation emitted as the nutrients ascended the stem. At the place where xylem and phloem had been separated a preference of paths was shown: namely, most, if not all, movement in an upward direction occurred in the xylem. This experiment seems direct and conclusive for conditions where at least moderate transpiration exists.

The fate of minerals as they are swept upward by a rapidly moving transpiration stream might be varied according to the mineral element, the plant, and the conditions existing within the plant at the moment. There are several possibilities, enumerated as follows: 1) a portion of the material will be captured by the cells adjacent to the xylem, in particular the cambium and young phloem; 2) a portion may move laterally via rays and the like to actively metabolizing cells; 3) a portion may be deposited in the leaves having moved there via the transpiration stream; or 4) a portion may move directly to the apical primordia and adjacent regions of active metabolism.

In a general sense there are two basic phenomena which influence the direction of movement of minerals within a plant. These are metabolic use and transpiration. The intensity of these factors in any tissue will determine the net movement to the tissue. The metabolic use of an element establishes gradients responsible for a continued flow.

Transpiration delivers to xylem extremities dissolved materials in proportion to the amount of water lost from the tissue. It should be remembered that the capture and metabolic use of elements from the transpiration stream may continually alter the mineral composition within the transpiration stream as it moves upward and, hence, the mineral composition of solutions delivered to the extremities of the xylem system may differ from that which begins its movement in the root region.

A characteristic distribution of radiophosphorus (P^{32}) to leaves of all ages on bean plants is shown in Figure 2. It can readily be seen that uniformity of delivery is more characteristic of older leaves than of younger ones. We have been unable to assign relative weights to the two factors, transpiration and metabolic use, in the determination of the quantity of P^{32} which will move to a given tissue, but it can be seen that the highest relative concentration invariably occurs in the region of highest growth rate. A carefully controlled microclimate around various meristematic tissues might aid in the further solution of this problem.

DEPOSITION OF MINERALS IN LEAVES

So far we have assumed that no chemical or physical reactions occur in the xylem system which might precipitate or absorb mineral components of the transpiration stream. I wish to discuss this possibility and to present positive evidence bearing on this point. Precipitation reactions in the xylem would alter the composition of the mineral solution available at the xylem extremities. Nutritional unbalance may then result. This condition could be further accentuated by a more rapid removal of water than of minerals (all or certain ones) from the transpiration stream.

Olsen (8) has presented some evidence that there may be a precipitation of ferric phosphate in the veins of leaves of the corn plant under certain growth conditions. This seems entirely possible. The phenomenon occurs on root surfaces where the epidermal cells begin the initial accumulation of minerals from the nutrient medium. Here, actively metabolizing cells are acquiring materials from a solution surrounding them. These materials cross the cortex and are "secreted" into the xylem

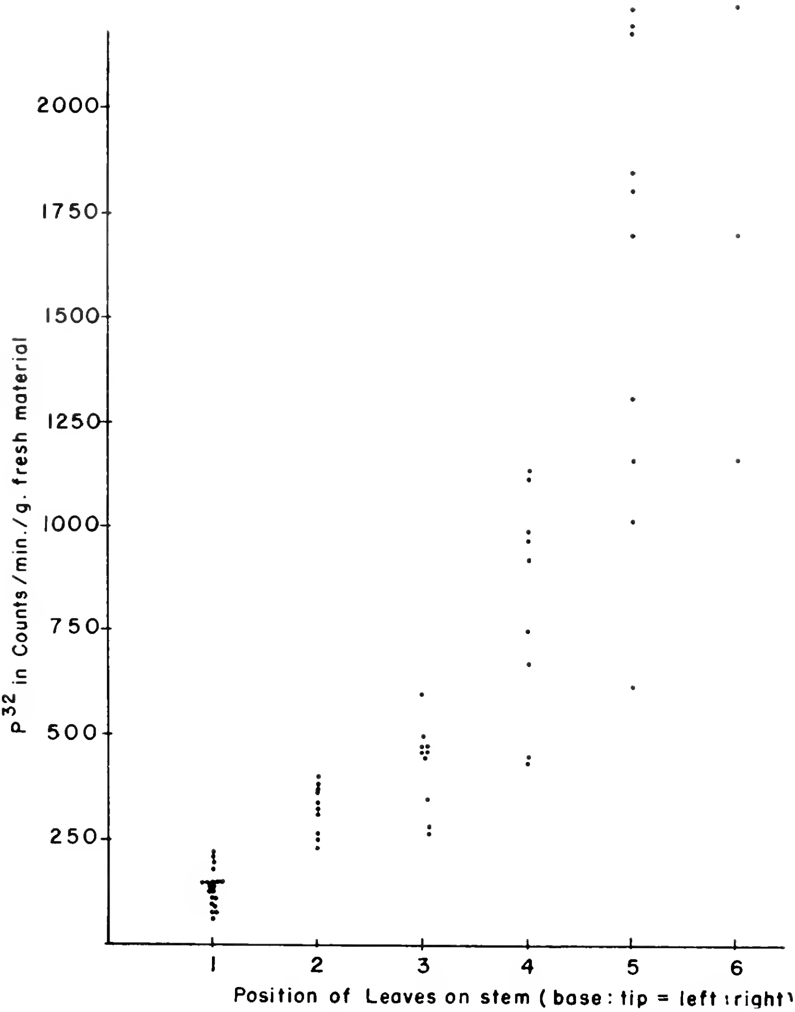

FIGURE 2. The amount of radiophosphorus acquired plotted against the position of the leaves on the stem for ten bean plants grown in the same nutrient tank. Oldest (opposite) leaves on extreme left; youngest leaves on right.

where they ascend via the transpiration stream to the xylem extremities. At these extremities the actively metabolizing cells acquire minerals from a solution which they in turn surround. In other words, two separate absorption processes must occur before the mesophyll cells of leaves acquire minerals, one by the cells at the root surfaces and the other by the cells surrounding the xylem. There is no valid reason to assume that precipitation reactions would not occur in the xylem extremities except perhaps that they may be partially protected by precipitation reactions occurring at the root surfaces and so may be partly "screened" from the possibility of reactions of similar intensity. Even so, precipitation reactions can be duplicated in both places. Our evidence is presented as follows.

Exactly comparable studies of precipitation reactions at root-solution and vein-mesophyll junctions cannot be made for anatomical reasons; therefore, two methods of study are necessary. The root-solution junction has been studied by conventional tracer methods, while the vein-mesophyll junction has been studied by autoradiography. Results are limited to iron and phosphorus. The uptake and translocation of iron by bean plants was measured by the usual colorimetric methods. The roots were not washed prior to analysis and so contained most of the iron which had accumulated both in the roots and on the root surfaces during the growth period. Figure 3 (lower part) shows the iron concentration of bean roots when grown for 12 days in solutions of various iron concentrations. The upper part of Figure 3 shows the distribution in the leaves of radioiron which was placed in the nutrient solution during two additional days of growth. These data show that the presence of a large amount of precipitated iron on the root surfaces has inhibited the uptake of additional iron ($Fe^{55, 59}$). The composition of the precipitate on the root surfaces has been analyzed and is known to be predominantly ferric phosphate at pH's below 6.0. At pH 6.0 some calcium is present while at pH 7.0 calcium is an important constituent along with the iron and phosphorus. In the nutrient solution used, the phosphorus-to-iron ratio was approximately 10:1.

With regard to the entry of phosphorus into the plant, the following experiment will show that it is influenced by the presence of iron in the nutrient solution. In this study the phosphorus-to-iron ratio was approximately 1:1. Figure 4 shows that as iron and, simultaneously, phosphorus increase on the roots, movement of phosphorus into the top is impaired. This, with the foregoing experiment, furnishes substantial evidence that precipitation reactions may occur on roots which may influence the ready entrance of iron and phosphorus into the root, and, therefore, the plant as a whole.

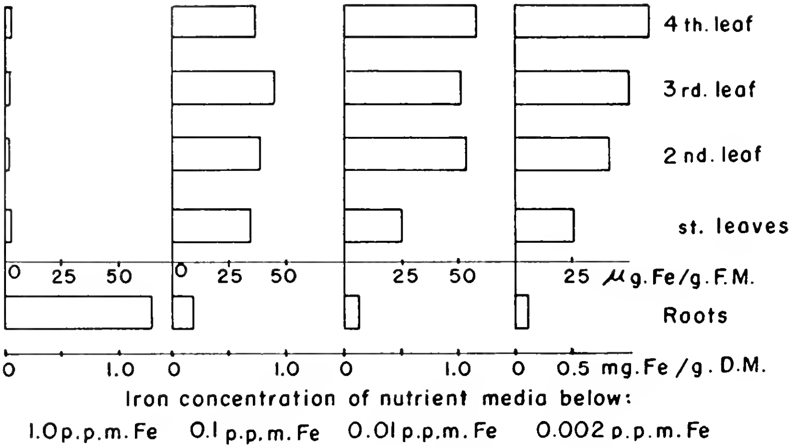

FIGURE 3. The amount of radioiron moving into the various leaves on four bean plants each grown at different iron concentrations and having different amounts of iron associated with the roots.

The most direct evidence that mineral nutrients may concentrate in veins and become immobilized has been attained for iron. Fe^{55} autoradiographs on no-screen X-ray film furnish a particularly good medium with which to show this phenomenon. Autoradiographs were prepared of bean plants grown at (*a*) pH 4.0 and medium phosphorus (0.0001 *M*), (*b*) pH 7.0 and medium phosphorus (0.0001 *M*), and (*c*) pH 7.0 and high phosphorus (0.001 *M*). Condition *a* results in healthy green plants. Condition *b* yields plants with chlorotic mesophyll, later becoming generally chlorotic. (The autoradiographs were made at the time that the tissue adjacent to the veins developed chlorosis). Condition *c* results in general chlorosis throughout the leaf (Figure 5).

The distribution of Fe^{55} in leaves under the above conditions shows: rapid entry and equal distribution throughout at pH 4.0 and medium

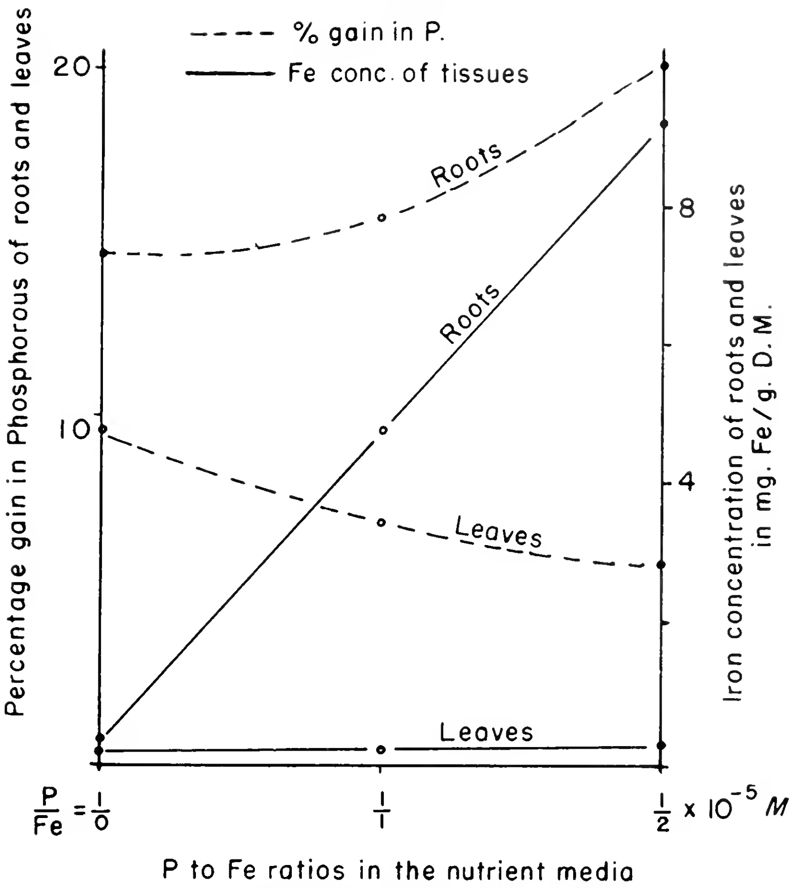

FIGURE 4. The percentage gain in phosphorus by parts of bean plants when grown in nutrient solutions 0.00001 *M* in phosphorus but with various levels of iron.

phosphorus; rapid entry into the vein system at pH 7.0 and medium phosphorus, but little or no distribution into the mesophyll. This accumulation of iron in the veins is extremely striking and indicates almost complete immobility. At pH 7.0 and high phosphorus the Fe^{55} fails to enter the xylem system due to its precipitation at the root surfaces, so there is little Fe^{55} in either veins or mesophyll. The results herein reported indicate that at pH 7.0 and medium phosphorus, relatively high concentrations of Fe^{55} may build up in the veins of the leaves. We are unable to determine whether the iron accumulates because of failure of the mesophyll cells to accept it as rapidly as the root cells accepted it or whether a preponderance of anions of one or more species resulted in precipitation. We suspect the latter and offer as partial proof the pattern of distribution of P^{32} in the leaf. It also is present in greatest concentrations along the veins (Figure 6). The presence in the veins of a precipitate such as ferric phosphate might be explained on the basis of more rapid withdrawal of water than of minerals, resulting in a concentration beyond the solubility limits of the least soluble salt (ferric phosphate at low pH's or a similar complex with calcium at pH 6.0 or above). The role of hydrogen ions in this phenomenon seems important, but, in considering them, we must remember the results of Arnon and Johnson (*1*) who have shown that the pH of "expressed sap" of leaves is not altered by the pH of the nutrient solution in which the plants grew. From these results it would seem, then, that the plasma membranes of the root cells are at least a partially effective screen against the ready entrance of hydrogen and hydroxyl ions. In the nutrient solution these ions play an important role in determining solubilities. They would play a similar role in the vein extremities if they reached these areas. We find evidence of precipitates on root surfaces and in vein extremities under particular growth conditions. These precipitates are essentially absent in both areas at pH 4.0 and occur in both areas at pH 7.0. It would appear that some of the hydroxyl ions are penetrating the root membranes and influencing the vein extremities even though they are insufficient to overcome the buffer effect of the cell sap. This is our present view. We see no great dissimilarities between the cluttering up of the root surfaces with insoluble materials and the similar apparent accumulation of insoluble materials in vein extremities.

RE-EXPORT OF MINERALS FROM LEAVES

Leaves differ in their basic metabolism according to age. In young leaves the predominant effect is the synthesis of new protoplasm which results in growth toward maturity. In older mature leaves, photosynthesis is the dominant function and little growth results. Carbohydrate ex-

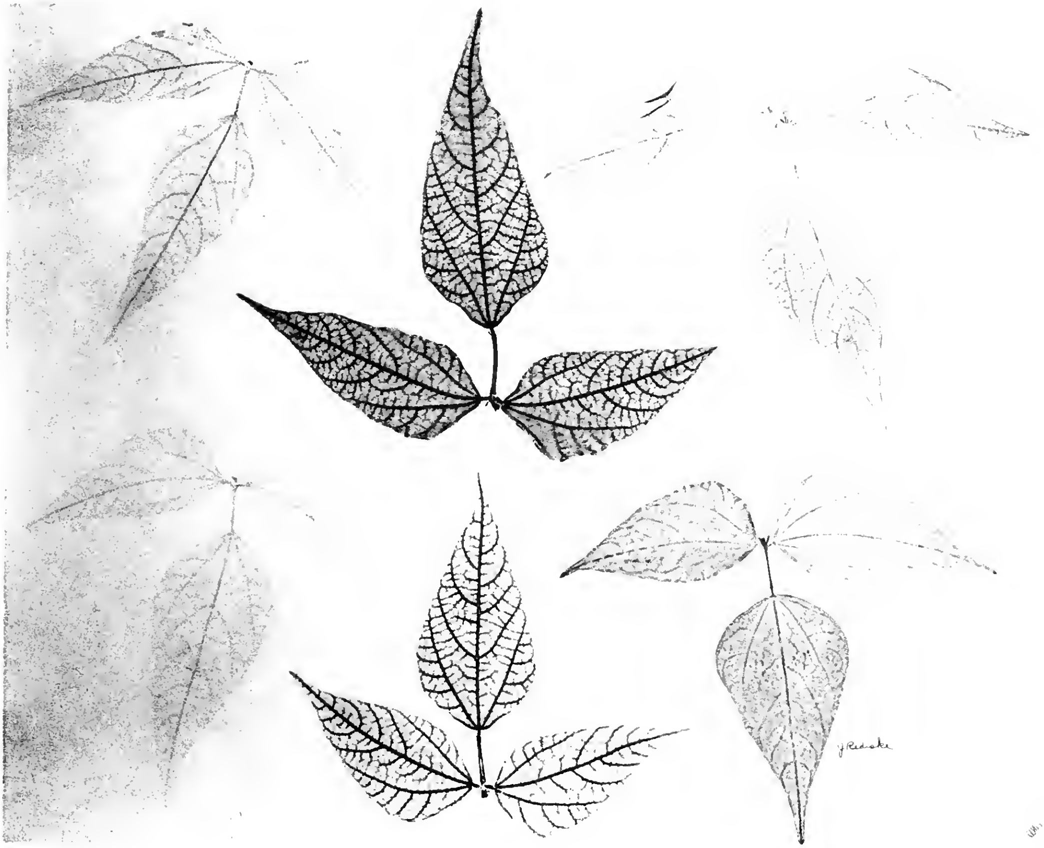

Figure 5. Autoradiograph of leaves of three separate bean plants receiving radioiron while they were becoming chlorotic as a result of the pH and phosphorus concentration of the nutrient media. *Left:* Plant grown at pH 7.0 and 0.001 M P. The leaves were chlorotic throughout. *Middle:* Plant grown at pH 7.0 and 0.0001 M P. The leaves had green veins with chlorotic mesophyll. *Right:* Plant grown at pH 4.0 and 0.0001 M P. The leaves were healthy and green throughout.

port to younger, actively growing tissues is an important part of their activities. The fate of the mineral nutrients arriving at these two widely different metabolic areas will differ. In young leaves metabolic use will tend to maintain a gradient in favor of continual movement toward these areas. The minerals acquired will be incorporated into new protoplasm. But what of the mature leaves where transpiration is high and metabolic use comparatively low? The transpiration stream will continually make available at the xylem extremities of leaves a supply of minerals. If removal of minerals from the xylem to the mesophyll were as rapid as delivery via the transpiration stream, there would undoubtedly occur an overshooting of metabolic needs in at least the mature leaves. This material must then be subject to re-exportation in order that continued transpiration may not result in unduly high mineral concentrations. Radiophosphorus moves readily into mature leaves and yet concentrations remain below those of the young tissues near the apical meristems. Competition for re-exported minerals for metabolic use within meristems furnishes an opportunity for movement and, provided the mineral in question is mobile within the phloem tissues, a pathway exists for its movement. The older mature and actively photosynthesizing leaves are located between two areas of active growth (roots and stem tips), and apparently supply both with some exported minerals. Since both areas have direct supplies available to them, the amount may be relatively low. However, it can be observed that exported phosphorus and iron reach both growing areas in sufficient quantities to be easily measurable.

Two experimental procedures have been employed to trace export of minerals from leaves. One involves the direct injection of a suitable form of a radioactive isotope into a vein of a leaf and the subsequent following of the migratory material (2). The other method involves the application of a radioactive isotope to the nutrient medium for a period of approximately 4 days, which allows sufficient time for the isotope to be well distributed within the plants. Half of the plants are then removed for autoradiographs and analyses, while the remaining half are placed in a normal nonradioactive nutrient solution for approximately 4 more days. During this latter 4-day period considerable new growth will have occurred; partly at the expense of previously acquired nutrients as the presence of the radioisotope in the new growth will attest. Autoradiographs and analyses are again made and carefully compared with the previous autoradiographs and analyses. Results obtained with phosphorus show that this element is withdrawn from the older tissue and moved to the meristematic areas where it accumulates in relatively high concentrations. The extent of the movement of previously acquired P^{32} into currently growing stem-tip tissue is shown in Figure 7. It will be seen that roots, stems, and lower leaves have lost considerable phosphorus to the stem tips. This indicates a rather free mobility of P^{32} in the "circulatory systems" of the plant.

The direct injection of a suitable form of a radioisotope into a vein of a leaf located midway up a stem will give a picture of the rapidity and direction of movement (2). Using this method it has been found that a diurnal trend in the movement of phosphorus from mature leaves is evident in bean plants. The most rapid movement occurs in the daylight hours with a strong downward movement extending to the root system in the late afternoon. Considerable movement in an upward direction to leaves higher on the stem and to stem tips frequently occurs. However, the extent of movement in an upward direction has proved to be more variable than the extent of downward movement.

This injection method has been coupled with the stripping method of Stout and Hoagland (9) previously mentioned, except that the stem was "stripped" immediately below the point of divergance of the leaf which was chosen for injection. Using this method with cotton plants, Biddulph and Markle (3) have attained results which show the phloem to be the tissue through which the P^{32} moved in leaving the leaf.

A quantitative estimation of the importance of leaf export of minerals to other areas as compared with the direct delivery from the root cannot be made without a more detailed study. Under certain artificial conditions, namely, leaf injection in low salt plants, it appears that movement is rapid enough to render this an important source of minerals to growing areas (Figure 8). It may be inferred that the re-export of mineral salts from leaves to roots may have a minor effect on salt uptake by lessening gradients between root cells and the external nutrient. While the effect of this factor may be measurable, it is difficult to see how it can be an important one.

FIGURE 6. Autoradiograph of parts of a bean plant receiving radiophosphorus via the nutrient solution.

The ultimate fate of downward moving phosphorus in the phloem, if it is not used metabolically, is undoubtedly to move up again through the xylem. The rapidly moving transpiration stream serves as a vehicle for transport of those ions which reach it by diffusion across adjoining tissue (6, 7). Biddulph and Markle (3) have shown a slight relative

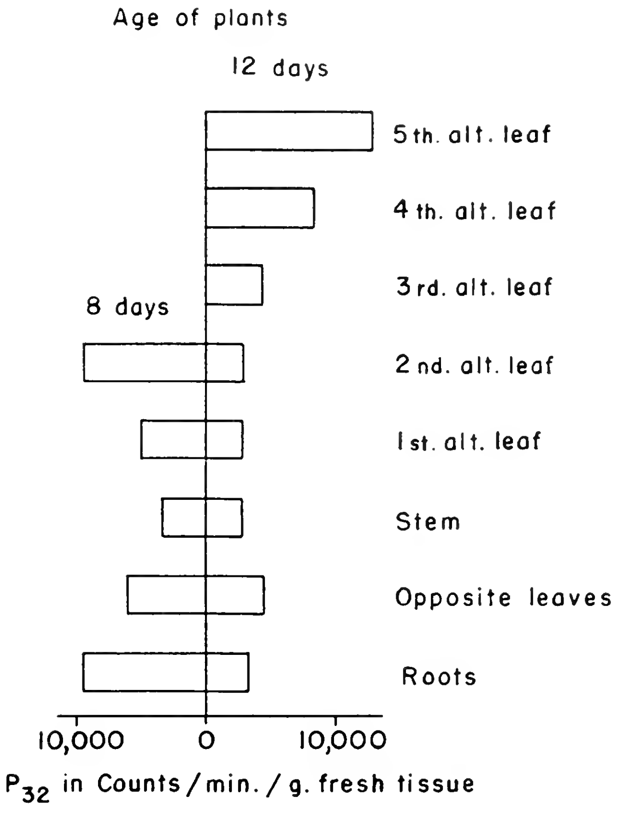

FIGURE 7. Movement of radiophosphorus into parts of bean plants. The plants to the left of the vertical line received P^{32} between the fourth and eighth days of growth; they were then harvested. The plants to the right of the line received P^{32} between the fourth and eighth days, but none from the eighth to the twelfth day; they were harvested on the twelfth day. The occurrence of P^{32} in the third, fourth, and fifth leaves is at the expense of that formerly present in the older organs.

gain in P^{32} within the xylem in ascending the stem from base to tip in cases where the radioisotope entered the stem from an injected leaf.

The status of re-export of mineral nutrients from leaves via the phloem system is that of an equalizing mechanism which comes into play when the transpirational factor in mineral distribution results in an "overshooting" of the requirement for metabolic use within the the particular tissue or organ, or when an impaired flow from the roots renders the meristems dependent on the existing supply within the plant organs. Movement of minerals in the phloem system makes it possible for the meristems to continue to receive and use minerals from other parts of the plant, withdrawing them where solubility and mobility permit. Elements like phosphorus, whose deficiency symptoms occur first in the lower leaves, appear to be freely mobile in the phloem and, consequently, the older tissues may continue to furnish the element to the meristem until they become depleted to a level below that required for normal metabolic functions. Conversely, the meristems are among the first tissues to suffer deficiencies when calcium, boron, iron, and perhaps others are involved. This means that withdrawal from older mature tissues does not constitute an important source of these elements to the meristems. In other words, these elements are not mobilized and translocated fast enough to avert a disaster in the meristems.

The study of translocation of minerals in plants is indeed in its infancy. It is an important study and should merit careful attention, for, frequently, it would appear that symptoms of malnutrition are as often due to failures in the distribution system as to failures in the initial absorption. Chlorosis is certainly in this category and in economic importance it ranks high among those factors responsible for decreased yield of economic plants. Radioactive and separated stable isotopes furnish tools which are extremely versatile and offer unique advantages in the study of translocation problems. Likewise, the biochemical processes making up the over-all metabolic patterns can be studied piecemeal by means of these tools until they yield the secrets of the forces which obtain in the orderly sequence of events constituting the life process.

Figure 8. Autoradiograph of a bean plant grown at pH 4.0, 0.0001 M P and 0.002 p.p.m. Fe. The radioiron was introduced into the leaf (via a vein of the leaflet showing the highest concentration of radioiron) 24 hours before harvest.

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SOME FIELD PROBLEMS IN PLANT NUTRITION

CHAPTER

11

Control of Nitrogen Effects on McIntosh Apple Trees in New York*

DAMON BOYNTON

It has been recognized for many years that nitrogen is more apt to limit the productivity of apple orchards under sod culture than is any other nutrient. It has also been realized that apple trees receiving heavy dosages of nitrogenous fertilizer may produce fruit of markedly less desirable color and quality than trees receiving less nitrogen. The McIntosh apple, which is now the second most important variety in the United States, is particularly responsive to nitrogen fertilization, and shows both of these nitrogen effects to a marked degree.

TREE RESPONSES TO NITROGEN FERTILIZATION

An illustration of the average effects of three levels of spring nitrogen fertilization on the yield and fruit color in a New York McIntosh apple orchard is given in Table I. In this orchard, total yield was significantly increased by the nitrogen increment from 0.5 to 1.0 pound but not by the increment from 1.0 to 1.5 pounds. The average percentage of the fruit sample grading fancy† was significantly decreased, however, by both increments of nitrogen. If it is assumed that the fruit sample from the outside of the tree represents the entire tree and that the total yield represents the yield of picked fruit, the product of the two should indicate how much fancy fruit was produced by the trees under these

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*In the course of the work discussed I have had a good deal of help and encouragement from my colleagues and assistants in the Pomology Department at Cornell University. In particular, I am indebted to Drs. A. J. Heinicke, M. B. Hoffman, R. M. Smock, and E. G. Fisher of the department, and to three graduate students, J. A. Cook, Richard Harris, and O. C. Compton.

†Fruits with half or more of their surfaces colored red.

treatments. This product is smallest for the highest nitrogen dosage. Actually the picture is not quite as simple as the table indicates because there was a greater drop of fruit from the high nitrogen trees than from the low nitrogen trees, average fruit color on the high nitrogen trees was not as well represented by the color samples as was the color from the low nitrogen trees due to the greater shade within the former trees, and the average size of the fruit was considerably smaller on the low nitrogen trees than on the high; but correction for these things does not change the picture greatly. Therefore, the data raise a question as to the need for a dosage of nitrogen more than 0.5 pound per tree in order to produce conditions satisfactory for maximum yields of high quality fruit in this orchard.

TABLE I

Average Yield and Percentage of Fancy Fruit from a New York McIntosh Apple Orchard under 3 Levels of Nitrogen Fertilization, 1942–1947

Annual Nitrogen Dosage per Tree		Annual Total Yield per Tree	Average Percentage of Fruit Sample Grading Fancy†	Column 2 × Column 3
H	1.5 lb.	15.0 bu.	54.0	8.1
M	1.0	14.8	70.2	10.4
L	0.5	12.7	81.1	10.3
LD*	5%	1.1	7.6	
	1%	1.4	10.2	

*Least difference for statistical significance at the odds indicated.

†50 apples were picked at random from the outside of each tree at harvest time. Fruits having half or more of their surfaces colored red were graded as fancy. The average percentage attaining this grade for the 16 samples in each nitrogen treatment is represented in Column 3.

That 0.5 pound of nitrogen per tree applied annually in the spring is a deficiency dose, in terms of the vegetation of these trees, is indicated clearly in Table II. It is apparent that there was marked increase of growth in the trees receiving the first, and again in the trees receiving the second, increment of nitrogen. The first increment was associated with increased total yield but the second was not. Nitrogen effects on the total leaf surface of a tree and its efficiency are cumulative. On a single shoot the number of leaves and their average size are directly

TABLE II

Girth Increase and Shoot Growth in a New York McIntosh Apple Orchard under 3 Levels of Nitrogen Fertilization

Annual Nitrogen Dosage per Tree		Average Girth Increase (1943–47)	Average Shoot Growth (1942–47)†
H	1.5 lb.	3.55 cm.	27 cm.
M	1.0	3.05	24
L	0.5	2.71	21
LD*	5%	0.41	
	1%	0.55	

*Least difference for statistical significance at the odds indicated.

†Twenty shoots per tree were measured at random after terminal growth had stopped in each year.

correlated with the length of the shoot, as is indicated in Table III. Not only were there the most leaves and largest leaves per shoot on the highest nitrogen trees, but the leaves contained the most chlorophyll and the two-year-old growth produced the most branches and spurs. Thus, the failure of the second increment in nitrogen to cause an average increase in yield was not due to a lack of vegetative response to the nitrogen, but resulted from failure of the vegetative response to be translated into yield response as it was in the case of the first increment.

Figure 1 shows that the annual yield fluctuations in this orchard were far greater than the differences associated with nitrogen level in any one year. These fluctuations from year to year were due largely

TABLE III

Correlations Between Shoot Length, Leaf Numbers, and Leaf Area for 120 McIntosh Apple Shoots from 2 Nitrogen Fertilizer Experiments, 1948

Correlation	*r*
Shoot length × number of leaves	0.918*
Shoot length × total area of leaves > 2 sq. in.	0.886*
Shoot length × average area per leaf of leaves > 2 sq. in.	0.525*

*Significant at 1 per cent point or beyond.

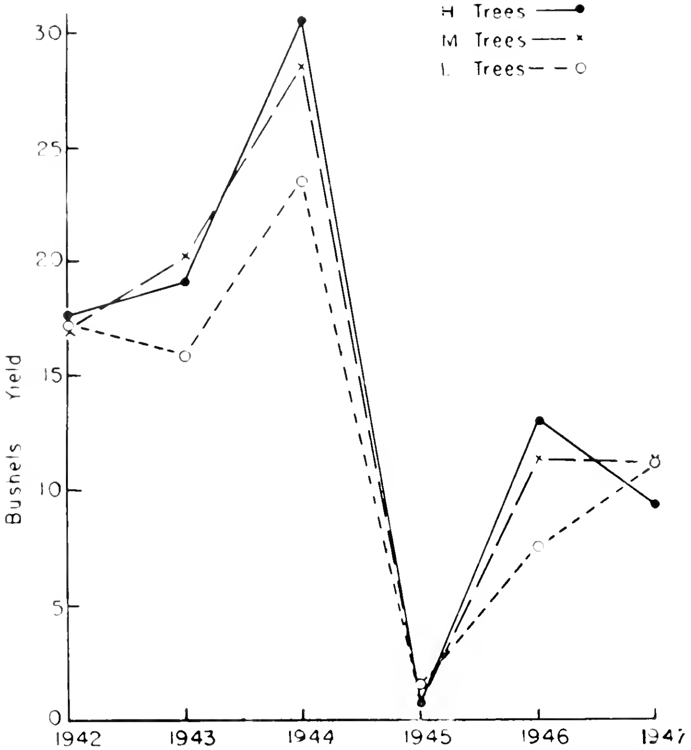

FIGURE 1. Average annual yield per tree in a New York McIntosh apple orchard under three levels of nitrogen fertilization. Annual treatments: H trees, 1.5 lb. N; M trees, 1.0 lb. N; L trees, 0.5 lb. N.

to the weather conditions in the period of bloom. In the six years, 1944 was the only one in which there was a long period of ideal weather for pollination, and no frost. In 1945, crop failure was the result of frost and cool rainy weather during the entire period of bloom. The years of 1946 and 1947 were less favorable for pollination than 1942 and 1943, although there was some opportunity in all four years. Thus, weather during the bloom was the most significant factor limiting yield in this orchard. In 1943, 1944, and 1946 the total yields of the low nitrogen (*L*) trees were significantly less than those of the intermediate nitrogen (*M*) trees. The direct causes of the decreased total yield due to nitrogen deficiency were decreased bloom and set, and smaller size; all of these were probably the result of the decreased leaf surface due to rather extreme nitrogen deficiency. Under the circumstances, even though no increase in the amount of fruit grading as fancy occurred on the average due to the first increment of nitrogen, this increment was justified. Had conditions been favorable for set in 1945, the light bloom which occurred on the low nitrogen trees in that year would undoubtedly have caused a markedly smaller crop than that on the *M* and *H* (high nitrogen content) trees. There is no such justification for the second increment in nitrogen. While there was growth response to it, the added growth was never associated with total yield response and it was always associated with decrease in fruit color.

Figure 2 shows that there was also fluctuation from year to year in the color of the fruit samples. It should be noted that there was a nitrogen effect on fruit color even in 1942 when there was no effect of nitrogen on yield, and that in 1944, the year of greatest crop, the average fruit color was poorest. The fluctuations from year to year

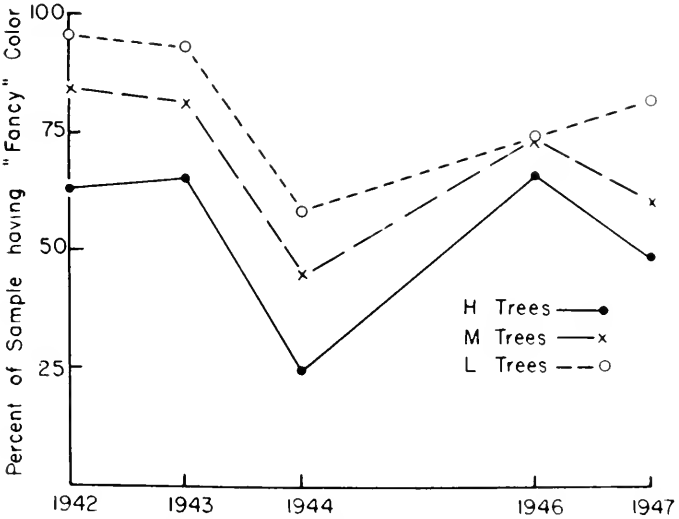

FIGURE 2. Average percentage of fruit samples having fancy color in a New York McIntosh apple orchard under three levels of nitrogen fertilization. Annual treatments: H trees, 1.5 lb. N; M trees, 1.0 lb. N; L trees, 0.5 lb. N.

seem to have been related to the weather conditions at the harvest period in the years of moderate crop. Cool bright weather at harvest time was conducive to the best color development. In addition, the very heavy crop in 1944 may have operated to reduce fruit color development in that year.

PRACTICES AFFECTING NITROGEN SUPPLY

These data should serve to illustrate some of the complexities of the problem of controlling nitrogen effects on apple trees with the purpose of obtaining maximum production and fruit quality.

The growth phenomena that are most important in determining yield occur largely in the early part of the growing season while fruit color and quality develop at the end of the growing season. Thus, a relatively high nitrogen status of the tree, favoring high yield, is desired in the early growing season, and a relatively low nitrogen status, favoring fruit color and quality development, is desired in the latter part of the growing season.

In the northeastern United States, weather most often is a dominant factor that limits crop size and quality. The grower cannot control the weather; but he can manipulate certain cultural factors, the most pertinent of which are 1) nitrogen application—material, rate, time, and method; 2) ground cover; and 3) pruning and spraying practices.*

Nitrogen application

Readily available inorganic or simple organic forms of nitrogen are used for ground application in McIntosh apple orchards in preference to forms which release available nitrogen over a long period of time. When moderate applications of such materials as ammonium nitrate, sodium nitrate, or ammonium sulfate are made in the fall or early spring, most of the soluble nitrogen is usually absorbed by plant roots before midsummer. At high rates of application, and with sparse ground cover or under heavy mulch, considerable available nitrogen may remain in the soil throughout the summer. Under such circum-

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* In the ensuing discussion it is assumed that nitrogen is the only limiting nutrient, that soil moisture and aeration are satisfactory, and that no unusual condition such as winter injury, trunk girdling, or insect or disease troubles have taken on major proportions.

stances it is not possible to control the nitrogen effects. Even when rather low rates of nitrogen application are made, the period of time that soluble nitrogen persists in the soil will vary greatly with weather conditions. Heavy winter rainfall following fall fertilization may in one year cause all applied nitrates to leach beyond the rooting zone, whereas following a winter of low rainfall all the nitrates applied during fall may be available for absorption. Because of this fact and because occasionally serious winter injury to the trunks of trees occurs following fall fertilization, it has been customary to apply nitrogen fertilizer in the early spring in New York. Late spring and summer applications are not made because they may inhibit fruit color formation and do not furnish available nitrogen at the time it is used in vegetation, fruit set, and initiation of flowers for a subsequent crop.

The most common method of ground application has been to broadcast in a ring under the branches of the trees. This has developed as a result of the fact that there is less use of the fertilizer by grass if it is applied in a concentrated band than if it is broadcast over the entire orchard floor. There has been little critical evaluation of this method of application, however, and it is possible that it is not the best under all circumstances. Certainly, the grass cover over the orchard floor deserves some attention and it may be worth while to fertilize it occasionally if not annually.

The use of urea sprays has recently attracted attention as a method of fertilizing McIntosh apple trees. Originally suggested in 1942, it has been employed commercially by apple growers rather widely during the past season. Table IV summarizes the results of some comparisons between ground fertilization and urea sprays made on McIntosh apple trees in 1948. These and other studies indicate that urea spraying is at least as effective as equivalent rates of ground application, in terms of yield, growth, and fruit color and size. The ultimate value of this method of nitrogen fertilization will probably be determined by the efficiency of direct absorption by apple leaves. Preliminary studies indicate that half the urea adhering to the leaf surface may be absorbed by the leaf within a few hours of application. Since as much as half of the spray material applied to the leaf may drop off, it is possible that frequently no more than a quarter of the urea applied to the leaves

TABLE IV

Comparison of the Effects of Sprays and Spring Ground Applications of Urea on the Yield, Growth, and Fruit Characteristics of McIntosh Apples in 1948. Average of Three Experimental Plots in Western New York

Urea Treatment		Av. Total Yield per plot	Av. Percentage of Fruit Sampling Grading Fancy	Wt. Fruit Sample (60 fruits)	Av. Terminal Growth†	Spurs Setting Fruit‡
N Pounds per tree	Methods of Application					
0 lb.	Ground, April	13.9 bu.	82.8	14.6 lb.	18.5 cm.	26.2%
0.6	Ground, April	14.6	66.3	15.4	23.1	27.1
1.2	Ground, April	16.2	56.3	16.2	26.4	31.1
2.4	Ground, April	18.5	40.4	16.6	28.5	32.1
1.0	Spray Calyx, 1st, 2nd covers	19.0	59.0	16.3	26.1	32.6
1.3	2 pink, calyx, 1st cover	17.7	55.6	16.6	28.0	33.1
LD* 5 per cent		1.8	8.5	0.6	2.6	2.0

*Least difference for statistical significance at odds of 19 to 1.

†Twenty shoots per tree were measured at random after growth had stopped.

‡The percentage of bloom and set on at least 200 vigorous spurs on 4 sides of each tree were determined. Since practically all spurs bloomed in 1948, the percentages are based on the total number of spurs counted.

enters them, the rest going to the ground where it is ultimately absorbed by roots. There appears to be far less efficiency in absorption by the upper surface of an apple leaf than by its lower surface, and young leaves seem to be more efficient than older ones. So it remains to be seen whether or not this development can be used to improve our control of the nitrogen nutrition of apple trees.

Ground cover

The presence of ground cover may be indirectly important in permitting control of nitrogen effects on apple trees. Nonleguminous sod uses large quantities of nitrogen in its growth and can be relied on to remove soluble nitrogen from the soil solution during the latter part of the growing season. This means that fertilizer practices in apple orchards should be designed to encourage grass growth. In New York apple orchards, grass responds most often to nitrogen and liming. Usually sparse grass growth in the areas between the trees is due to lack of nitrogen. Failure of cover growth from lack of calcium and magnesium occurs under the trees where acidification by sulfur sprays has been most rapid. While there is no experimental substantiation of this idea, it is probably worth while from the standpoint of nitrogen control alone to fertilize for healthy grass growth in McIntosh apple orchards.

The use of supplementary mulching materials spread under apple trees is a common practice in many New York apple orchards. When these mulches are composed of materials high in carbon, like straw or nonleguminous hay, their initial effect is to depress the available nitrogen supply in the soil. This effect may be apparent for a year or more in apple trees mulched with high carbon materials. If the mulch blanket is maintained over a period of years, ultimately nitrates will build up under it and will remain high throughout the entire year. Thus, heavy mulching even with nonleguminous materials causes a loss of control of nitrogen effects. There has been no critical study of moderate mulching in relation to nitrogen fertilization, but it is probable that if annual mulch additions are light enough so that grass grows through the blanket freely, satisfactory nitrogen control may be maintained.

Pruning and spraying practice

Pruning and spraying practices may have important indirect influences on the nitrogen requirements and responses of apple trees. Heavy pruning has been repeatedly demonstrated to act in the same way as nitrogen fertilization, and in orchards where pruning is severe, the nitrogen fertilizer program may be somewhat curtailed in order to improve fruit color without loss of productivity.

Nitrogen fertilization, to a considerable extent, has been used as a means of overcoming the detrimental effects of fungicides on vegetation of apple trees. It was a common experience for apple growers changing from lime-sulfur to elemental sulfur fungicidal spray programs to find that it was desirable to reduce the rate of nitrogen fertilization. Recently, an experiment reported by Dr. D. H. Palmiter has indicated that elemental sulfur sprays may also reduce vegetation and that nitrogen fertilization may offset these effects. He found that over a six-year period apple trees sprayed with Fermate (ferric dimethyl dithiocarbamate) and given no nitrogen fertilizer yielded as much as trees sprayed with elemental sulfur and given 5 pounds of urea annually. Both of these treatments resulted in an increase in yield of 38 per cent or more over the yield of trees unfertilized and sprayed with sulfur. While there was some nitrogen in the Fermate spray, analysis of leaves indicated that the increase in yield could not be attributed to that; rather, it seems to be due to absence of spray injury.

OTHER CONDITIONS AFFECTING NITROGEN RESPONSES

There are, of course, a large number of conditions that may limit nitrogen responses besides the ones that have been discussed. In New York McIntosh orchards, excess or deficiency of soil moisture and serious injuries to the leaf surface from insects, disease, or spray materials are not uncommon limiting conditions; occasionally potassium and magnesium deficiencies may be significant factors. Their effects on nitrogen responses are of several sorts. Excess moisture in heavy soils results in poor soil aeration which may both reduce the absorbing surface of the root system and the ability of the absorbing surface that is there to function efficiently. This will ultimately lead to the death of the tree. Most commonly, the periods of poor aeration occur in the spring and are short enough to permit survival of mature trees in marginal locations; the effect is one of general devitalization and failure to respond to good management in growth and productivity. Soil moisture deficiency, occurring usually in midsummer or later, may have no influence on growth or set of fruit, and may actually result in increased bloom the following spring. However, the size of the fruit in the year of drought is decreased in proportion to the duration of the period of moisture unavailability in the soil. Fruit color may be somewhat improved as a result of some moisture deficiency but, if the deficiency period is prolonged and continues until harvest, the quality of the color may be poor and preharvest drop tends to be very heavy. Injuries to the leaf surface from insects, diseases, spray materials, or mineral deficiencies modify nitrogen responses in different ways, depending on the time and severity of loss of effective leaf area. Early damage or defoliation such as that caused by severe primary infections of apple scab or lime-sulfur injury, may reduce vegetative growth, fruit set, and flower initiation for subsequent crops. Later leaf injuries, such as those caused by mites, by leaf hoppers, by arsenical injury, or by potassium or magnesium deficiencies may have little or no influence on growth, set, or subsequent crop, but tend to decrease fruit size and quality and to predispose the trees to heavy early fruit drop.

DIAGNOSTIC OBSERVATIONS ON NITROGEN RESPONSE

These, then, are some of the main practices which the grower may manipulate in the attempt to control nitrogen effects on the McIntosh apple tree and some of the other conditions that influence nitrogen responses. Under the variable weather conditions of the northeastern United States, it seems unlikely that very close control will be possible but some measure of it may be obtained by adjusting practices according to careful diagnostic observations. Because of the perennial nature of the apple tree, these diagnostic observations need to take into account separately: tree responses to conditions of the current season and tree responses to conditions of previous seasons.

The first and most important observation to be made is an evaluation of the crop on the tree, in relation to the bearing capacity of the tree and the bloom, and in relation to the best average fruit quality that is practically attainable. Next, measurements of shoot growth, leaf size, and leaf color appear to be particularly useful in diagnoses of this sort. These are interrelated more frequently than not but furnish in combination useful quantitative evidence of the nitrogen status of an apple tree. Table V illustrates the effects of four rates of nitrogen fertilization on some of these measurements on shoots from two New York McIntosh apple orchards. For such measurements to be useful under field conditions, they have to be easily made and to be referred to a set of growth standards. Leaf color may be determined by reference to color standards for apple leaves now available or may be made from

TABLE V

Average Leaf Number, Leaf Color, Leaf Size, and Shoot Length of Representative One-Year-Old Terminal Growths from Two McIntosh Apple Orchards Under Differential Nitrogen Fertilization in 1948

N Applied Annually per tree	Average Leaf Chorophyll	Average Shoot Length	Average Total Leaf Number for Leaves > 2 sq. in.	Average Area per Leaf (sq. cm.) of 4 Largest Leaves
	mg./65 sq. cm.			
0.0 lb.	1.42	16.5 cm.	10.2	32.7
0.6	1.75	21.2	11.7	35.5
1.2	1.96	28.4	14.7	42.5
2.4	2.32	31.2	16.0	41.2

alcohol extracts of leaf disks. Leaf size is easily and accurately determined by measurement of length and width and by reference to conversion tables previously prepared. These measurements may be rated on the basis of reference groupings like those in Table VI.

Terminal growth of shoots and development of leaves in number and size are determined to a considerable extent by the food reserves in the tree at the beginning of the growing season. Leaf color, on the other hand, reflects more closely the nitrogen supply in the tree at the time of observation. Thus, evidence of the effects of food reserve and of the current nitrogen supply may be obtained from these four

TABLE VI

Tentative Growth Standards for New York McIntosh Apple Shoots and Leaves

Standard	1 (low)	2	3	4	5 (high)
Shoot length, cm.	< 15	15–20	20–25	25–30	> 30
Number leaves > 2 sq. in. per shoot	< 10	10–12	12–14	14–16	> 16
Size largest leaf, sq. cm.	< 30	30–35	35–40	40–45	> 45
Leaf color (July 15–Aug. 15) Chlorophyll per 65 sq. cm. surface	< 1.6	1.6–1.8	1.8–2.0	2.0–2.2	> 2.2
McIntosh leaf color standards	1	2	3	4	5 and >

measurements. If shoot length, leaf numbers, leaf size, and leaf color are all abnormally low or abnormally high, it is likely that both the food reserve and the current nitrogen supply were abnormally low or high. If growth, leaf numbers, and leaf size are low and leaf color is high, it is likely that food reserve was low but that the current nitrogen supply has been increased. If growth, leaf numbers, and leaf size are high and leaf color is low, then it is likely that the food reserve was considerable but the current nitrogen supply is not great enough to maintain color. Determination of leaf color at intervals throughout the growing season may give additional diagnostic evidence of the course of current nitrogen supply. Figure 3 shows the chlorophyll trends and total nitrogen trends in leaves from a McIntosh apple orchard under six nitrogen treatments, involving different levels, times, and methods of application. In all cases the midseason peaks of the curves are determined by the total rate of nitrogen application: the higher the rate, the higher the peak. The peak for treatment C was reached later than for the other treatments; this was associated with the fact that three of the six urea sprays were applied after the middle of June. All of the chlorophyll curves tended to flatten at the end of the growing season more than they do in many years. This may be due to the fact that the orchard was harrowed several times in July in order to eliminate deep ruts created by the heavy spray machinery, a practice which may have released nitrate in midsummer and checked the normal sod cover at the end of summer. Yields were low in this orchard due to poor pollination and there were no significant differences among them, but fruit color was inversely related to nitrogen in all cases. It would seem, from this and other studies, that the seasonal chlorophyll curve associated with highest yield potential and best fruit color would be rather high in early summer and rather low at harvest time. The leaf color range of 1.8 to 2.0 mg. per 65 sq. cm. surface, in July, reflects a compromise between these two opposing objectives. Of course, interpretation of such measurements must be based on experimental nitrogen response studies over a period of years in the climatic zone where the diagnosis is needed. This is because the nitrogen responses of an apple variety differ in different zones as well as from year to year. Whereas the range of group 3 in Table VI seems to be correlated with satis-

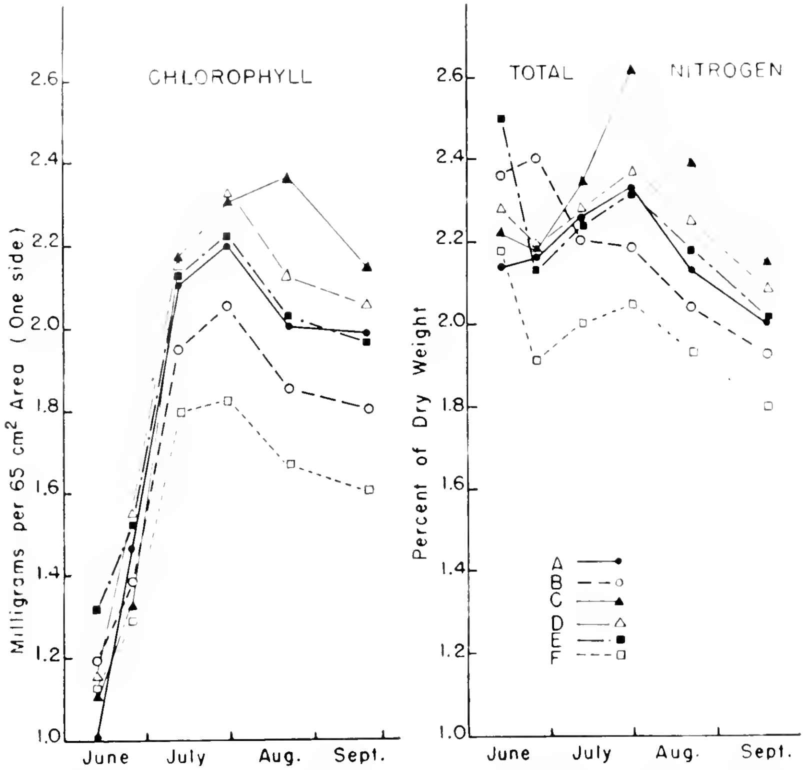

FIGURE 3. Leaf chlorophyll and nitrogen in leaves from McIntosh apple trees under various urea spray and nitrogen fertilizer treatments in 1947. The treatments were as follows:

F 0.0 lb. N ground
B 0.6 lb. N ground
E 1.2 lb. N ground

A 1.2 lb. N—3 sprays
D 1.2 lb. N—3 sprays + 0.6 lb. ground
C 2.4 lb. N—6 sprays

factory average nitrogen responses of McIntosh apple trees in Western New York, in the Champlain Valley some of the growth standards of group 2 may be more closely correlated with the most satisfactory nitrogen responses. It also seems possible that under the high light conditions of the McIntosh producing area in British Columbia, the measurements of groups 4 and 5 may be correlated with the best nitrogen balance.

Since nitrogen responses may be modified by so many things other than the nitrogen supply, critical systematic evaluation of the most likely of the modifying influences is frequently essential to a complete understanding of the nitrogen response of a McIntosh apple tree in a particular year. This is especially important when it is necessary to unravel the complex of possible reasons for failure of the tree to respond to nitrogen in the way that somebody thinks it should. Why did it grow more or less than expected? Why did it bloom in this year and not that? Why was the fruit large or small in spite of this or that? Why was the fruit quality better or worse than anticipated? These questions often cannot be answered without specific knowledge, not only of the temperature, light, and moisture conditions of the previous two growing seasons, but also of the internal drainage and moisture reserve of the soil, the insect, disease, and spray injuries to which the foliage of the tree was subjected, and the status of the tree with respect to nutrients other than nitrogen. The more exact this knowledge is, the more easily special limiting conditions can be sorted out of this group of possible ones. While there is still much to be done, steady progress has been made in the understanding of these complicating factors and in the recognition of diagnostic criteria for them.

Thus, the success with which a New York McIntosh apple grower copes with the problem of controlling the nitrogen effects on his trees will vary from year to year depending on weather, and other conditions beyond his control; but, on the average, accuracy in diagnosis and skill in manipulating the cultural conditions in the orchard will point him toward maximum yields of the highest quality fruit. It is the business of the pomologist to help him with such practical problems in plant nutrition, and I have tried in this paper to outline briefly the general approaches that we are using in New York.

CHAPTER

12

Production of Vegetable Crops for the Canning Industry*

JACKSON B. HESTER

THE MONETARY VALUE of the vegetable crops primarily used in the canning industry has assumed tremendous proportions. For example, approximately 600,000 acres of tomatoes are grown in the United States, a considerable proportion of which is used by the canning industry. Assuming the approximate average yield as five tons per acre, this crop compares with the monetary value of all of the wheat grown in the North and South Atlantic States. This, of course, does not take into consideration the monetary value of other vegetable crops such as carrots, beans, peas, cauliflower, and many others.

The amounts of plant nutrients consumed by these crops are enormous and of great economic importance. In the production of the tomato crop alone, the plants absorb approximately 13,650 tons of calcium, 2250 of magnesium, 16,050 of nitrogen, 1500 of sulfur, 5100 of phosphoric acid, 30,000 of potassium oxide, 68 of iron, 98 of manganese, 8 of boron, 12 of copper, and 18 tons of zinc. Considering the different climatic and soil conditions under which these crops are grown, scientific and practical problems of great significance are encountered. This article will be largely the consideration of those practical and scientific problems encountered in the production of vegetable crops in the various sections of the United States as observed by the writer.

TOMATO PLANT PRODUCTION

About two billion plants are required for 600,000 acres of tomatoes.

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* Because of the general and more practical nature of this paper, literature citations have been omitted.

Many different systems of acquiring these plants are used. In the East and Middle West the majority of the plants are transplanted with field grown plants from southern Georgia. Between eight and ten thousand acres of land are devoted to the production of plants in southern Georgia. These plants are grown, for the most part, on the Tifton sandy loam soil in the counties of Tift, Grady, Colquit, and Lowndes. Plant production and transporting to the northern markets is a painstaking and nerve-racking job.

First, the soil is a leached semilateritic type with a pH value between 5.0 and 5.5. The replaceable calcium and magnesium are extremely low, ranging between 100 and 300 pounds per acre for calcium and near 100 pounds for magnesium. Fertilizer is as essential for the growth of plants as seed. In other words, to leave the fertilizer out of the row is equivalent to not planting the seed. The soils are subject to extreme leaching and during the plant growing season it is not uncommon to have as much as 10 inches of rainfall within one week. Considering the importance of fertilizer and the possibility of excessive rainfall, the maintaining of sufficient plant nutrients in the soil to mature a satisfactory plant within six to eight weeks of time becomes an operation of significant importance.

Most of the acreage of tomatoes in the northeastern and middle western states is transplanted within a period of three to four weeks. Therefore, the Georgia grower has an extremely narrow period of time in which to plant, produce, and ship hundreds of millions of plants. These plants are pulled between the stages of 6 and 9 inches in growth, wrapped in bundles of 50 with peat moss which is often supplied with nutrients to be utilized by the plants during shipment. Every grower realizes that unless he produces a satisfactory plant for shipment he will be unable to fulfill his contract. Therefore, owing to the poor nature of the soils in respect to the plant nutrient requirements of tomatoes, careful consideration is given to fertilizer application.

For the most part, 600 to 800 pounds of a specially prepared 4-10-6 (nitrogen-phosphorus-potassium) fertilizer mixture are used. This fertilizer is made physiologically neutral with finely ground dolomite limestone. Owing to the prevalence of "white-bud" or zinc deficiency in corn in southern Georgia, 10 pounds of zinc sulfate per ton of fertilizer and borax, at a similar rate, are added.

One hundred and twenty-five thousand plants are expected to be pulled from each acre of ground. A chemical analysis of these plants reveals that they contain approximately 6.4 pounds of calcium, 2 of magnesium, 10 of nitrogen, 3 of phosphoric acid, 13 of potassium oxide, less than 1 pound of iron, and approximately 0.2 pound of manganese. The total dry weight is about 400 pounds. It is therefore obvious from the amount of fertilizer applied that the plants utilized only a small proportion of it. This is undoubtedly due to mechanical distribution, unpulled plants left in the field, and leaching.

An ideal plant for shipment is 6 to 9 inches high and analyzes approximately 90 per cent water, 2.3 per cent nitrogen on the dry weight basis, 0.8 per cent phosphoric acid, 3.9 per cent potassium oxide, 1.6 per cent calcium, 0.5 per cent magnesium, 235 parts per million of iron, and 40 parts per million of manganese. The chemical composition of the plant is extremely important. Plants that carry too much water or excessive concentrations of nitrogen are soft and do not ship well nor live after being transplanted in the fields.

Plants are also grown in Nevada, Texas, Tennessee, and Virginia. In Texas and Nevada the pH value of the soil is approximately 8.0. The soil carries tremendous quantities of calcium, magnesium, potassium, and sodium, but is deficient in nitrogen, phosphoric acid, and minor elements like manganese and iron. The problem, however, becomes not so much one of plant nutrition as of supplying water in sufficient quantities and getting the plants to sufficient size to ship at the proper time.

Many local plants are grown in hotbeds, greenhouses, and open fields. It is sufficient to say that the problems encountered in the growing of Georgia and Texas tomato plants are also involved in the growing of local plants in each territory. While this is in no way a full account on tomato plant production, it does serve to point out the different problems involved in plant production and their importance to tomato production.

TOMATO PRODUCTION

The plant nutrient requirements of a large yield of tomatoes are very great. The average yield of tomatoes in the eastern part of the United States over a ten-year period, 1935 to 1944, was 5 tons per acre.

However, it is of significant importance to note that 20 tons per acre are not uncommon and yield records in Pennsylvania fields have exceeded 30 tons per acre. The average yields since 1940 have been considerably higher than 5 tons per acre. Since the average yield is so much lower than the possible yield, it becomes a challenge to condition all soils to at least raise the average yield if not to accomplish the highest yield with all growers. Under most conditions the lack of plant nutrition is the limiting factor, although in certain years disease is a definite factor. However, if a satisfactory crop is not grown, which is true in many cases, the use of spray for disease control is of no avail.

Careful analysis of many fields and plants has revealed that approximately 100 pounds of nitrogen, 200 of potassium oxide, 35 of phosphoric acid, 93 of calcium, 15 of magnesium, 10 of sulfur, and a few ounces of iron, manganese, copper, and zinc with small amounts of boron and molybdenum per acre are necessary to produce for harvest 10 tons of tomatoes. This is a considerable amount of nutrients and is not supplied by the average soil without supplemental applications of fertilizer materials. The amount of supplemental application of fertilizer materials varies according to the soil types and farming practices in the various localities. Practically none of the leached soils of the eastern seaboard has enough plant nutrients to grow 10 tons of tomatoes without supplemental applications.

The largest need of fertilizer occurs in the soils of the Eastern Shore of Maryland and Virginia. These soils have been notoriously low in average yields over a long period of years. The soil fertility problems vary from farm to farm and must be handled individually, but the actual deficiencies occur in approximately the following order: (*a*) The soil reaction or pH value, for the most part, is below 5.5. Soils are extremely low in calcium and magnesium and high in readily soluble aluminum. This makes the question of liming of prime importance and the use of dolomitic limestone necessary. (*b*) The soils are low in organic matter and total nitrogen supply, making the second problem of importance the provision of the necessary amount of nitrogen. (*c*) The available potassium content is low and the total supply is low, making it necessary to apply potassium for each crop. (*d*) The supply of boron also is low, and if lime and fertilizer are applied in sufficient amounts to produce over 6 tons of tomatoes per acre, this element is likely to become deficient. Consequently, our fourth problem is that of supplying an adequate amount of boron without producing toxicity. This is done by the use of approximately 10 pounds of borax per acre. (*e*) Owing to the fact that these soils have been acid so long, much of the native supply of manganese has come into solution and been leached out. Manganese becomes a limiting factor particularly when the soils are limed to an optimum condition for the growth of tomatoes. (*f*) Fertilizer materials with large amounts of phosphoric acid have been used on these soils for so many years that it is only in exceptional cases that a response from phosphorus is obtained. Some of these soils, in the plowed area, have as much as twenty times the original supply.

The same problems that occur in the aforementioned sections also occur in New Jersey. However, in the case of boron, response to the application will probably result after a yield of 7 to 8 tons rather than the 6-ton yield previously mentioned.

When you move into a section like Pennsylvania, however, the situation changes. Instead of calcium and magnesium becoming a limiting factor, phosphorus is the number one problem. This is because the principal crops grown in the past have been grain, corn, and sod crops in which a limited amount of fertilizer has been used. Many of these soils are derived from limestone and, although liming is necessary in many cases, it is not the limiting factor in plant growth. So, owing to the high fixing power of many of the red soils, phosphorus must be added. The second problem is nitrogen and potassium, followed by lime and the minor elements. Boron is the most important minor element that becomes deficient and it is not likely to show up in the Piedmont belt soils until a yield of 10 or more tons per acre is realized. It might be noted that copper and zinc have not been mentioned. The reason for this is that a spray program to control disease has been inaugurated in which the alternating sprays are a zinc compound and a copper compound. This seems to have been sufficient to take care of these two deficiencies under all conditions.

Conditions in the Midwest and Canada are like those in Pennsylvania: phosphorus is the first limiting factor, and next are nitrogen and potassium. Here again, the yield must be 15 tons of tomatoes per acre before a response to boron is noted. In one experiment in Canada no increase in yield with borax was observed until sufficient nitrogen, phosphorus, and potassium were added to produce a yield of 15 tons. Then 10 pounds of borax per acre produced 3 additional tons of tomatoes. The pH values of the Canadian soils are practically all within the optimum range for the growth of tomatoes or above the optimum range. This statement, of course, applies only to those regions in which tomatoes are primarily being grown, that is, near Chatham, Toronto, and the lake border.

The principal limiting factor in Canada is the length of the season. For that reason, getting the plants started early in the spring is very important and controlling the vegetative growth is equally important. Owing to the heavy nature of the soils, the long winters, and the wet springs, phosphorus is particularly unavailable. Therefore, response to phosphatic soil supplements is great. Oats, for instance, have been observed to be two weeks earlier on the heavily phosphated plats as compared to those grown on the regularly fertilized plats. Therefore, tomatoes mature earlier when adequate phosphate is supplied early in close proximity to the root zone.

A definite response to manganese and an improvement in the red color of tomatoes have been noted on the alkaline soils of Illinois.

QUALITY OF TOMATOES

The quality of the tomatoes is a determinate factor in the amount of tomato products sold. Two important factors influence the quality of tomatoes: climatic conditions and soil conditions. The climatic conditions influence the yield and quality throughout the season, but a cold, wet picking season probably has the most unfavorable effect. Analyses for three very different seasons serve to illustrate: a poor season (1938), a fair season (1939), and a good season (1943). These data are given in Table I. It is noteworthy how the total solids, sugars, titratable acids, and vitamin C increased due to better growing and picking conditions. In other words, the season, as it influenced the soluble plant nutrients in the soil, also influenced the quality of the fruit.

Soil type influences yield and quality of tomatoes. In 1939, tomatoes grown on four different soil types were investigated for yield and quality. These data are given in Table II.

TABLE I

Influence of Climatic Conditions upon the Quality (processed puree) of Tomatoes in New Jersey and Pennsylvania

Year	No. Samples	Total Solids, g./l.	Sugars, g./l.	Insoluble Solids, g./l.	Titratable Acids, m.e. liter	Ascorbic Acid, p.p.m.
1938	41	59.7	33.6	5.59	62	219
1939	80	63.6	34.4	6.04	64	230
1943	31	68.6	40.0	—	66	243

The Sassafras sandy loam produced the best quality if not the best yield—that is, more sugars, solids, and vitamin C. On the other hand, the Edgemont stony loam gave the poorest quality. The sugars were low, the titratable acids high, and vitamin C low.

SOIL FERTILITY FACTORS

The above-mentioned information shows that the quality of tomatoes grown on different soil types varies. It is believed that the quality of tomatoes, as influenced by soil types, is primarily a question of difference in soil fertility factors.

Soils with obviously abnormal soil fertility factors were selected and 25 pounds of Rutgers tomato fruit were taken from each field for the purpose of ascertaining the influence of fertility factors upon the quality of the crop. Chemical analyses of the soils from these fields are given in Table III. For example, the Adelphia sandy loam, a poorly drained

TABLE II

Influence of Soil Type on Quality (processed puree) and Yield of Tomatoes

Soil*	Soil Amendments Applied, lb./acre			Number of Samples	Yield, tons/acre	Total Solids, g./l.	Sugars, g./l.	Titratable Acids, m.e. liter	Ascorbic Acid, p.p.m.
	N	P_2O_5	K_2O						
A	37	117	60	9	9.12	66.7	40.0	58	280
B	35	110	70	9	7.56	58.2	34.9	56	225
C	26	82	52	9	11.27	54.1	28.8	62	221
D	20	82	41	9	8.64	52.7	25.0	74	194

*A—Sassafras sandy loam, 1939
B—Sassafras loam
C—Penn silt loam
D—Edgemont stony loam

soil, was selected for the study of the influence of drainage upon quality. In New Jersey this soil type is often planted with tomatoes, but seldom produces a bumper crop due to water injury. Judging from the organic matter content, this soil is in a state of high fertility for the type. However, as shown in Table IV, the yields were very poor.

At this point, it is necessary to mention that the given analyses of fruit are representative of those obtained, since a complete presentation of the data would serve only to confuse rather than to clarify the subject. For comparison, typical data of the analyses of soil and of fruit produced on a soil in a state of high fertility are given at the bottom of the table in each case.

The quality of the fruit from the Adelphia sandy loam was poor, and the yield, as indicated in Table IV, was very low. Undoubtedly, the high water table in the soil interfered with the soil processes and the normal function of the plant. The color of the processed fruit was abnormally poor, since it lacked the red coloring matter and was too high in the yellow pigment. Of the minerals for which analyses were made, calcium was the only one abnormally low, although the nitrogen content was below average.

TABLE III

Soil Conditions and Nutrients Applied

				Soil Analyses			Soil Amendments Applied, lb./acre				
Soil*	pH†	Organic Matter, per cent	N, lb. per acre	Ca	Mg	K	CaO	MgO	N	P_2O_5	K_2O
A	5.9	1.8	1,800	good	good	fair	100	15	60	108	84
B	5.7	1.0	1,000	fair	good	fair	740	0	25	40	35
C	5.45	1.7	1,700	fair	fair	poor	0	0	75	0	112
D	5.7	2.3	2,300	good	good	fair	160	30	40	160	0
E	5.0	1.2	1,200	poor	poor	fair	80	0	50	80	70
F	6.6	1.7	1,700	good	good	poor	150	20	40	120	80
G	5.7	2.3	2,300	good	good	poor	0	0	0	0	0
H	5.6	2.4	2,400	fair	good	good	870	480	37	185	135

*A—Poor drainage conditions, Adelphia sandy loam
B—Low organic matter content, Collington fine sand
C—Low pH value, Woodstown sandy loam
D—Low potassium content, Sassafras sandy loam
E—Low magnesium content, Sassafras sandy loam
F—Low manganese content, Sassafras sandy loam
G—Low fertility field, Sassafras sandy loam
H—Fertile field, Sassafras sandy loam
†pH on a 1:2 soil-water ratio (glass electrode)

The organic matter content of a soil is generally considered to be a measure of fertility. This fact is brought out by the analyses of the fruit from fields of low organic matter content. The yield of fruit was low and of poor color and it contained low percentages of sugars, potash, and nitrogen. Undoubtedly, available nitrogen is one of the constituents most likely to be lacking in a soil of low organic matter content. The analyses of a large number of fruit samples from plants known to be low in nitrogen have not revealed any particularly abnormal fruits. It must be recognized that if the available nitrogen in a soil becomes sufficiently low to produce a small and yellow plant, the foliage may be lost and the fruit indirectly spoiled from hot sunshine or other weather conditions. However, so far as can be learned from the analyses, no particular mineral constituent was greatly influenced by a low nitrogen content of the plant. The available phosphorus of soils has been shown to be influenced by the organic matter content. The analyses did not reveal that phosphorus greatly influenced any particular constituent of the fruit. However, a low content of available phosphorus in the soil delayed maturity, and fruits picked late in the year are likely to be poor. Of course, both available phosphorus and nitrogen greatly influence the yield and likewise affect the nitrogen and phosphorus content of the fruit.

Extreme soil acidity is a potent factor in soil fertility for certain crops. Here again, the yield seems to be the principal factor affected, although

TABLE IV

The Influence of Soil Fertility Factors Upon Quality of Tomatoes

Soil*	Yield, tons per acre	Color †	Ascorbic Acid, p.p.m.	Sugars, g./l.	Total Solids, g./l.	Ash, g./l.	Content, m.e./l.						pH of Puree	
							H	Ca	Mg	K	N	P	Plain	Salt
A	4.09	C	256	32.0	63.5	5.67	61	1.60	2.60	60.8	85	—	4.15	—
B	4.31	C	227	29.6	51.9	5.68	77	3.80	2.18	34.5	102	15	4.4	4.15
C	8.07	B	290	40.9	67.4	5.95	55	2.30	1.60	56.3	120	10	4.25	4.05
D	8.35	B−	196	34.1	57.8	6.60	55	3.80	1.10	38.4	122	10	4.25	4.05
E	7.56	C	265	28.4	59.0	5.30	96	2.80	0.70	62.4	105	9	4.1	3.9
F	7.12	B	193	25.1	48.0	4.84	72	3.00	3.04	26.3	113	15	4.2	3.95
G	3.22	C	219	32.2	56.0	5.73	54	4.20	1.40	37.6	123	10	4.35	4.25
H	10.68	A+	326	48.2	64.0	6.10	67	3.01	2.19	56.6	106	18	4.45	4.25

*The soils are the same as those identified in Table III.

†This and other properties based on processed puree.

the calcium and magnesium content was below that of fruit from more fertile fields. Acid soil conditions delayed maturity. Since high-quality production occurs early in the season, extremely acid soil conditions are to be avoided. However, certain constituents, such as available manganese and iron, are influenced by the acidity of the soil and may indirectly influence quality.

LOW AVAILABLE POTASSIUM AND MAGNESIUM

A number of quality factors have been attributed to low potassium content of the soil. The principal ones affected seem to be yield, ascorbic acid, sugars, and potassium content. The foliage on plants with potassium deficiency is rapidly lost, thus causing the fruit to be exposed to the elements. Yellow top fruits are much more prevalent on plants with potassium deficiency. The loss in processing is much greater than with normal fruit. The yellow in the tissue tends to destroy the brilliancy of the processed product.

Magnesium often becomes a limiting factor in tomato production on the eastern seaboard. The soil selected in this case was very poorly supplied with both calcium and magnesium. Eighty pounds of calcium oxide per acre were supplied in the fertilizer, but no magnesium was added. The plants on this field broke down very badly from magnesium deficiency. The fruit from these vines was poor in color and low in magnesium and sugars which is characteristic of this deficiency. The most important failing of a tomato plant deficient in magnesium is the breakdown of the foliage, leaving the fruit unprotected from hot sunshine.

BORON IN TOMATO PRODUCTION

Earlier in the production of tomatoes when the yields were comparably low, boron deficiency was no problem. However, beginning about 1940 when the yields of tomatoes had been considerably increased over the former yields, boron deficiency symptoms became increasingly evident. In fact, boron deficiency became the number one problem in tomato production in certain sections. Research work has established the fact that on the eastern shores of Delaware, Maryland, and Virginia, under average conditions, boron deficiency is likely to occur when the yield is above 5 to 6 tons per acre. It must be remembered that certain of these soils are sufficiently well supplied with boron to produce many more tons to the acre, and boron deficiency might even occur at lower yields on other soils.

Therefore, it becomes obvious that as the soil is limed to the proper pH values and as increased amounts of fertilizer are added and increased yields obtained, the deficiency of such elements as boron and manganese become increasingly important. Available manganese has been linked with the red color, sugar, and vitamin content of tomatoes. Here again the balance seems very important. For example, in this connection, 1000 pounds of a 3-12-12 fertilizer mixture seemed in balance on a heavy Brookston silty clay loam, whereas 2000 pounds produced an exceptionally nitrogen-deficient plant. In other words, the phosphorus and potassium had produced a sufficiently larger growth to make nitrogen a deficient element in this formula. This same thing has been clearly demonstrated in an experiment in New Jersey, using fertilizers with higher nitrogen content.

From the analyses it may be seen that the fruit from soils of high fertility is superior in almost every way. Such factors as brilliant luster, fine texture, and excellent taste are to be found in fruit produced on fertile soils.

The trade demands good products and will switch to other products; so it is all important to produce a product of such quality to maintain the market.

SCOTCH PEAS

The Scotch pea is a small, deep green, dried pea used in making green pea soup. It has a very desirable color and flavor. It is grown in sections of the country that have a very short growing season and long days. Roseau County, Minnesota, adjoining the Canadian border and the Lake of the Woods, produces several thousand acres of these peas. The soils in this section vary from sands to clays. One characteristic of the soils is high pH values which vary between 7.0 and 8.5. The calcium content is very high; in fact, the mean replaceable calcium, magnesium, and potassium content of 60 samples is as follows: 254, 60, and 2.79 milliequivalents per 1000 grams of soil.

During 1946 a large shipment of peas was unsatisfactory from the standpoint of cooking. The normal process is to soak the peas overnight, cook in an open kettle, pass through a cyclone to remove skins and hard peas, and then blend into soup. Many lots of the peas grown in 1946 would not cook normally. In fact, some of these peas continued to get harder instead of soft on cooking. Experimental lots cooked 24 hours were just as hard as at the beginning of the cooking period. Samples of soil and peas were collected for investigation. The data in Table V summarize this information. The Scotch peas were soaked overnight, cooked 30 minutes, and then the texturemeter reading was taken. As the calcium and magnesium content of the soils increased, the potassium decreased, and the peas became increasingly hard to cook. Lots of peas that were difficult to cook were electrodialized in the Mattson dialysis cell for 30 minutes, thus removing considerable calcium and magnesium, and then cooked. These became soft before the water boiled. When equivalent amounts of the extracted calcium and magnesium were added to the electrodialized peas in the form of chlorides, they again failed to cook properly. In the purchase of dried yellow peas in Canada, it has long been the custom to purchase them on the basis of their suitability for cooking.

TABLE V

Influence of Soil Conditions on the Cooking Quality of Scotch Peas

Scotch Peas Cooking Quality Texturemeter Reading	Replaceable Bases of Soil, m.e./1000 g. soil			
	Calcium	Magnesium	Total	Potassium
0–10	185	51	236	3.46
11–20	226	64	290	2.68
21–30	335	63	398	2.72
31–	271	60	331	2.29
*Fertile Sassafras soil	110	10	120	3.00

*This is a New Jersey soil for comparison.

Soils from Warroad, Minnesota, were put in 3-gallon coffee-urn liner pots in the greenhouse for investigation. Increased amounts of potassium were added to the various pots in combination with phosphate and certain minor elements. Peas grown on soil receiving 60 per cent muriate of potash at the rate of 400 to 500 pounds per acre showed a more pleasing green color and cooked more rapidly than peas without potassium. Experimental plots using the fertilizer formula 0-20-36 at the rate of 0, 200, 300, 400, 500, and 600 pounds per acre gave yields of dry peas as follows: 851, 985, 1046, 1212, 1118, and 1364 pounds per acre. However, the more pleasing thing was that the peas receiving the heaviest application of fertilizer had the best color and cooking qualities.

CANNING PEAS

Specific requirements are placed upon every crop used in the procedure of making soup. For instance, the peas used should be small and dark green. The No. 2 and 3's are more desirable than the larger peas. For a number of years peas have been grown in Pennsylvania and New Jersey. However, most of these peas have been used primarily as a frozen pack. No particular emphasis was placed upon the production of peas in Pennsylvania; consequently, the yields were relatively low. The average yield from 1928 to 1932 was approximately 1700 pounds per acre. However, the varieties planted were the large seeded type unsuitable for soup production.

An intensive research program was started to increase both the yield and quality of peas produced. Three factors were found to be limiting in the soil: (*a*) the soil reaction or lime status, too acid, (*b*) insufficient nitrogen, phosphorus, and potassium were being used for maximum production, and (*c*) improper methods of applying fertilizer were being employed.

Some of the soils on which attempts were made to grow peas were as acid as pH 5.0. These soils were largely the Penn silt loam, Lansdale loam, Bucks silt loam, and related types. However, many peas were planted on the Hagerstown silt loam which is a soil derived from limestone and has a pH value generally around 6.5. The initiation of a conscientious liming program improved the soils physically and made the conditions in the soil favorable for the proper use of fertilizer materials. The small type of pea (deep green Superlaska) was found to be most satisfactory. Using fertilizer mixtures such as 0-9-9, 3-9-9, 6-9-9, and 9-9-9 in various amounts and different rates of seeding varying from 3 to 7 bushels per acre, it was found that 1000 pounds of 9-9-9 fertilizer with 4 to 6 bushels of seed per acre gave the most satisfactory yield. However, the fertilizer analyses recommended to the growers were based upon the soil analyses for available nutrients and the organic matter content. Such fertilizers as 4-12-8, 5-10-10, and 7-7-7 were actually used. It was also found that the higher amounts of fertilizer tended to increase the number of large peas over the number of small peas. In other words, with 5 bushels of peas per acre seeded with no fertilizer, 38 per cent of the peas were Nos. 1 and 2 and 62 per cent were Nos. 3 and 4. On the other hand, with 1000 pounds of 9-9-9, 80 per cent of Nos. 3 and 4 and 20 per cent of Nos. 1 and 2 were produced.

Using the available farm equipment, namely, grain drills, it was found that the most satisfactory method of applying fertilizer was drilling the fertilizer at right angles to and immediately before seeding. It is significant to say that on several thousand acres of peas in 1947 to 1948, the average yield in Pennsylvania was more than 2 tons per acre.

LIMA BEANS

The most desirable type of lima bean for vegetable soup manufacture is the small seeded dark green bean. For maximum production of lima beans a soil with a pH value between 6.2 and 6.8 is desirable. One of the great difficulties in growing lima beans is to produce a good vigorous growing plant that will set a large part of the crop at one time. Weather conditions enter into this problem and the question of getting a good set of beans at any particular period is dependent, to a certain extent, upon the existing weather conditions. However, plant nutrition is a decided factor in this condition. It has been found that the plants that have a heavy set also have a high calcium content. Consequently, liming acid soils and the use of fertilizer mixtures high in phosphatic materials tend to supply large amounts of calcium to the plant. Cultural practices, such as proper methods of weeding to eliminate grass in lima bean production, are very important.

On one 2000 acre planting of lima beans on the Eastern Shore of Virginia, by properly correcting the calcium and magnesium content of the soil and the application of the most desirable type of fertilizer (such as 4-12-8, 6-8-6, and 5-10-5 carrying 10 pounds of borax per ton of fertilizer and used at the rate of 600 to 1000 pounds per acre depending upon the analyses of the soil), the yields were increased from less than 1000 pounds to more than 2000 pounds per acre.

These same conditions were encountered on the acid soils in Pennsylvania. In fact, without proper methods of fertilization and liming, some yields were as low as 150 pounds per acre.

CARROTS

The requirements for carrots are rather specific. It is very desirable to have a deep orange color in preference to some of the lighter colors of certain varieties. Large yields of high quality carrots are desirable for commercial purposes. The large yields make it possible for the grower to clear substantial funds from his planting, and the high quality raises the commercial potential. Fortunately, it has been found that with large yields high quality is usually obtained.

The application of certain chemicals for the control of weeds has greatly simplified the production of carrots. Carrots grow very slowly during the early stages; in fact, it was found that only 4 per cent of the plant nutrients ultimately absorbed by the plant was absorbed during the first 70 days after planting, 27 per cent was absorbed during the next 30 days, and 69 per cent during the remaining time. This, of course, means that abundant plant nutrients must be available to the plant during the latter stages of growth.

The carrot has also been found to require relatively large amounts of potassium. Consequently, the analyses of commercial fertilizer such as 5-10-10 and 4-8-12 have proven most desirable. Sidedressing methods have also proven very satisfactory. Where the yield exceeds 20 tons per acre, application of fertilizer up to and including one ton per acre has been necessary. It has been most desirable to have the land in a high state of fertility before planting carrots, that is, limed the year before and at the same time fertilized in part so as to increase the fertility of the soil.

When the use of chemicals to control weeds became so universally accepted, many of the growers neglected to follow through with the proper methods of cultivation. As a result, the soils became so compact at the end of the year that aeration became a problem and production was depressed. Experiments on cultivation clearly illustrated that proper methods of cultivation often mean the difference between success and failure in producing the crop.

SUMMARY

An effort has been made to point out the tremendous number of applications possible in field culture of the theory and practices of nutrition developed by the various branches of agricultural science. Many problems have been investigated and solved for practical conditions. A large number of problems in nutrition under field conditions remain unsolved. Undoubtedly, as new information regarding the availability of plant nutrients found in minerals and humus is supplied and as the different requirements of various plants are uncovered, many of these problems will be readily solved with a minimum of expense to the farmer and to industry.

ROLE OF MINERALS IN PLANT NUTRITION

CHAPTER

13

Growth and Function as Criteria in Determining the Essential Nature of Inorganic Nutrients

DANIEL I. ARNON

THE KNOWLEDGE of what inorganic elements are indispensable for the growth of green plants is obviously of fundamental importance in plant nutrition. Certain inorganic elements are essential for the life of all living forms, but, in the case of plants, inorganic or, as it has been traditionally known, mineral nutrition looms particularly large. Among the multitude of life forms, autotrophic plants are, except for certain bacteria, the only organisms capable of synthesizing all of their food requirements for maintenance, growth, and reproduction if supplied with the mineral essentials including water and carbon dioxide.

How can the mineral requirements of plants be determined? More specifically, what inorganic elements are essential for the growth of higher plants grown in soil? The present discussion will concern itself with this latter question. No attempt will be made to survey the kindred field of inorganic requirements of lower plant forms or of animals, except in so far as some considerations pertinent to green plants apply to other organisms as well.

The natural soil medium for the growth of higher plants is an extraordinarily complex physical, chemical, and biological system. Its suitability for plant growth is determined by a proper interaction of the various soil factors to make available to the plant a continuous and adequate supply of essential inorganic nutrients. To answer then the question, what specific elements are essential in plant nutrition, it is necessary to determine which of the elements derived from the soil are required for plant growth through the stage of reproduction. The simplest approach to this problem is an analytical one. An accurate analysis of the inorganic constituents of plants should give an inventory of the elements derived from the soil. This is indeed the case, but the list of elements derived from the soil, unfortunately, is not synonymous with the list of elements essential for plant life (*22*). The plant has the capacity, within rather wide limits, of indiscriminate absorption: it absorbs essential as well as superfluous or even harmful elements. Every essential element must of course be present in the plant, but not every element present is essential. It will be shown later how a failure to recognize this fundamental property of plants may lead to erroneous conclusions drawn from otherwise convincing evidence.

Since the composition of the plant offers no reliable guide as to the essentiality of the constituent elements, we find it more profitable to turn to the external medium in which the plant grows. An obvious approach is to remove from the external medium, one at a time, its constituent elements and to observe what effect this has on the plant. If the plant failed to grow as a consequence of removing element A but not element B, the conclusion would be drawn that element A is essential and element B is dispensable. This straightforward approach, however, is not applicable to the natural soil medium in which plants grow. To attempt a complete removal of an element from a soil would entail for most nutrients, a chemical treatment which would alter the soil from a natural to an artificial medium. Nevertheless, there are soils in nature which, for one reason or another, are deficient in plant nutrients. The addition of the deficient element would restore or enhance plant growth and thus provide a clue to essentiality.

Although it is common experience in agricultural practice to correct nutritional deficiencies by soil fertilization, the ubiquitous complexity of heterogenous soil components often complicates the interpretation of observed plant responses. The difficulty arises in attempting to distinguish between a direct and an indirect effect of a given soil treatment on the plant. In one case an element added to the soil is of direct benefit by virtue of its being absorbed by the plant; in the other case the benefit to the plant accrues from secondary changes in the soil brought about by the treatment. For instance, it was shown by Horner *et al.* (25) that vanadium has a beneficial effect on nitrogen fixation by *Azotobacter,* and Jensen (27) reported a similar effect on nitrogen fixation by *Clostridium.* It is thus conceivable that additions of vanadium to a soil low in nitrogen might be reflected in improved plant growth, but this cannot be taken as evidence that vanadium is essential for the growth of higher plants. Other instances of indirectly beneficial effects of soil treatments on plants are due to the ion exchange status of a soil where an application of one ion may, through exchange reactions, render another ion available for the needs of the plant (7). The exchange of one ion for another, as for example, calcium for sodium, may also favorably alter the physical condition of a soil and improve plant growth through better aeration and water penetration. It is likely that most of the "beneficial" growth responses from adding to soils inorganic elements not recognized as essential may be due to such indirect effects on the soil. (In some cases, however, a *partial* substitution within the plant of a nonessential element for an essential one, may be involved. This point will be treated later in the discussion).

Even when failure of plants to grow is caused by a single nutrient deficiency in the soil, it may be difficult to discover the missing element by soil treatment alone. A deficiency of an element in the soil may often be due not to the low concentration but to the unavailability of the nutrient to the plant. There are cases where the fixing power of the soil is such that relatively huge applications of an element are required to give a plant response even though the quantity needed by plants is very small. Treatment of the soil only, in the absence of information derived from other techniques, could therefore easily lead to erroneous conclusions. A case in point is afforded by a study of zinc deficiency in California (*17*). In a peach orchard showing zinc deficiency, it was computed from results of plant analysis that the trees and the fruit removed about 8 ounces of zinc in seven years, yet an analysis of the soil showed 3000 pounds of zinc to the acre within the root zone.

In another zinc-deficient soil, 1500 pounds of zinc sulfate applied to the soil failed to cure zinc deficiency in apples. It would be easy to miss the nature of the deficiency in these cases if treatment were confined only to the soil. On the other hand, the essential status of zinc was demonstrated by techniques of foliage spraying or tree injection. Though the soil was by-passed completely, a correct diagnosis and treatment was achieved. In California, these are indeed the practical methods of treatment of zinc and other micronutrient deficiencies in the field.

There seems to be a basis then for reaching the paradoxical conclusion, that the natural growth medium of land plants, the soil, is least adapted for the study of the indispensable nature of plant nutrients. It is with artificial nutrient media, water and sand cultures, that the essential status of the various elements found in plants and derived from the soil was established. The recent history of plant nutrition offers no case of a discovery of a new essential element through soil treatments. For every one of the micronutrients for example, evidence of their indispensability was available from nutrient solution experiments, well in advance of any responses reported from the field. The artificial culture technique continues to be a powerful and discriminating tool in evaluating the indispensability of inorganic nutrients in plant nutrition.

A question may be raised as to the soundness of applying conclusions drawn from nutrient solution studies to the growing of plants in soil. There seems little doubt that the availability and absorption of several important plant nutrients is different in soils than in nutrient solutions. There is likewise little doubt that the nutritional requirements of plants are the same in soil as in nutrient culture. It is not implied, of course, that the same elements will be absorbed in the same amounts from different media, be they a group of soils or a series of different nutrient solutions. The capacity of the plant to absorb nonessential elements was already mentioned. It is also well established that plants can grow in soils or nutrient solutions of varying compositions. It is difficult to conceive, however, that a different set of inorganic requirements would govern the growth and successful completion of the life cycle of a plant such as the tomato, depending on whether it was grown in soil or in water culture. The relative inorganic composition of the various organs remains essentially the same whether plants are grown in soil, sand, or water culture (5).

Until recently the physiological validity of artificial cultures rested on small-scale experiments with limited opportunities for appraising the crop-producing potentialities of this method of supplying nutrients to plants. Conclusive as the vast number of culture solution experiments was in establishing the physiological suitability of the method, there was until recently no direct comparison of the inherent productive capacity of a fertile soil with a favorable nutrient solution. A special study of this problem with the tomato as the test plant has revealed (6) that the capacity for crop production of sand and water culture media is of the same order of magnitude as that of a highly productive soil. The productivity of artificial nutrient media was even found under certain conditions to surpass that of highly fertile soil.

The wide use of nutrient solution techniques, which in recent years included a number of commercial ventures, has provided impressive evidence bearing on other fundamental aspects of plant growth in soils, namely, the importance of organic matter and soil colloids. A large number of species of higher plants has been grown successfully in artificial culture with the roots furnished only with a solution of inorganic salts, under suitable conditions of root aeration. No factor inseparable from the soil, nor any preformed organic compound externally derived, including vitamins (3), appears to be indispensable to the functions of the plants investigated. Under normal conditions these plants, which include most of the agricultural species, are fully capable of synthesizing the organic substances which they require. The importance of organic matter and of clay colloids in soil is of course beyond question, but these factors may be regarded as operating in a secondary way and should be distinguished from those factors that are indispensable for plant growth in a primary manner. It may be stated that the progress of plant nutrition in the current century has corroborated the fundamental concept of the inorganic nature of plant nutrition as developed in the nineteenth century by De Saussure, Liebig, and Boussingault.

One final point with regard to the physiological adequacy of nutrient culture techniques: Claims have arisen in some quarters that plants grown without organic matter are of inferior nutritive value as food to animals. These claims carry the implication that the metabolism of plants grown in an inorganic medium differs from that of plants grown in the presence of organic matter. Since nutrient solution culture represents an extreme case of an inorganic medium, plants grown in this way and subsequently fed to animals provide a means of testing these contentions. A preliminary investigation was therefore undertaken to compare the nutritive value of grasses grown in water culture and in a soil with a history of organic manuring, using the guinea pig as the test animal. No evidence was found that plants grown in an inorganic medium are deficient in any dietary essentials (8).

If the physiological validity of the nutrient solution technique is accepted, it becomes pertinent to examine its suitability in investigating what inorganic elements are essential for plant growth. The nutrient solution technique is obviously free from the chief inherent shortcomings of the soil. Individual nutrients can be added or omitted as desired. Synthetic media are prepared lacking one element at a time, but containing all the others accepted as being essential. The growth of plants is then observed. Failure to grow in the absence of the element and resumption of growth upon the addition of the deficient element are taken as evidence of essentiality. It was substantially with this approach that the classical list of essential elements was compiled in the nineteenth century. Exclusive of carbon, oxygen, and hydrogen, the list included seven elements derived from the soil: nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, and iron.

Even in the early days of the use of the nutrient culture technique, observations were made by different workers of the "stimulating effect" from adding elements to the nutrient medium other than the seven recognized as indispensable. But there was no consistent basis for considering them essential. Circumstances were not propitious for advances in this direction. The salts which were used as sources of the seven nutrients contained various impurities unknown to the experimenter. Water, even of the distilled kind, and containers in which the plants grew, served as other sources of impurities. There was little in the experience of the research workers in those days to enable them to anticipate the low order of concentration of the newer plant nutrients, now known as micronutrients. Despite these obstacles the concept of a micronutrient, an element which though used by the organism in minute amounts is nevertheless essential for life, was formulated with remarkable insight as early as 1869 by Raulin (*40*), who discovered the indispensability of zinc for *Aspergillus niger,* and considered this element essential for higher plants as well. He explained growth without the addition of zinc as being due to the presence of zinc as an impurity in the medium. This point of view was further elaborated at the turn of the present century by Javillier (*26*), who found that zinc was present as a contaminant not only in the purest chemicals then available on the market but was also derived from the glass used for containers. Strong support for the essential nature of nutrients required in minute quantity by higher plants was also provided during this early period by the work of Mazé (*37*), who used corn as the test plant.

Important and fundamental as these concepts of the French workers proved to be, they were not generally accepted even in the country of their origin, where other investigators claimed that the effects were not specific (*34*). Only with the evolution of careful procedures for the removal of accidental impurities was decisive evidence obtained for the essentiality of micronutrients for plant growth. The careful work of Steinberg (*47*) provided confirmation for the early thesis of Raulin that zinc is essential for *Aspergillus niger.* An extension of the purification techniques to other elements combined in some cases with a felicitous choice of test plants resulted in the demonstration that zinc and three other elements—boron, copper, manganese—are essential for higher plants as well (*35, 53, 45, 44*). The biological significance of these findings was reflected in the fact that species differing so widely taxonomically and physiologically as *Aspergillus niger* and the tomato were found to share the requirements for zinc, manganese, and copper. The practical significance of these discoveries was soon attested by a vast and ever-growing list of hitherto obscure diseases of crops grown in the field which were identified as micronutrient deficiencies (*48*). The latter development has incidentally provided once more a demonstration of the fitness of the nutrient solution technique in elucidating problems of plant nutrition in soils.

In the early part of the thirties of the present century, the evidence was strong that in addition to the seven elements on the classical list, four others derived from soil—boron, zinc, copper, and manganese—are to be included among the nutrients essential for the growth of higher plants. There was a feeling of uncertainty, however, whether the list was complete even with the addition of these four elements. The very circumstances of their previous obscurity and newly-acquired importance raised the question whether other impurities are not likewise being overlooked. These considerations have moved D. R. Hoagland to prepare several so-called A–Z solutions, containing a rather large number of elements in minute quantities which were to be used in supplementing the standard nutrient solutions. In an experiment with strawberries, Hoagland and Snyder (*23*) gained the distinct impression that plants receiving an enlarged A–Z solution, containing 22 elements in addition to the four micronutrients, were superior to all others. This observation, although strongly suggestive, did not lend itself to quantitative evaluation. Soon afterwards in an investigation of the relative merits of ammonium and nitrate as sources of nitrogen, it was found that molybdenum, chromium, or nickel improved the growth of barley plants in a culture solution supplied with ammonium salts as the sole source of nitrogen (*1*).

These observations seemed to justify the undertaking of a systematic investigation to test the hypothesis that the list of micronutrients, then confined to boron, manganese, zinc, and copper was incomplete. This was done by arranging a number of elements in groups and by observing how the addition of a given group affected the growth of plants in culture solutions (*2*). Three supplementary solutions, each containing different elements in minute quantity, were prepared. One solution, designated A4, furnished the recognized four micronutrients, boron, manganese, copper, and zinc. The basic culture solution, supplemented with the A4 solution, therefore furnished the plant with a seemingly complete list of essential elements. Another supplementary solution, designated B7, contained the following seven elements: molybdenum, vanadium, chromium, nickel, cobalt, tungsten, and titanium—a somewhat arbitrary grouping based on the consideration that each of these could assume various valency levels and hence, conceivably, participate in oxidation-reduction processes within the plant cell. The already mentioned findings on the role of metals in the nitrogen nutrition of barley (*1*) suggested this particular grouping. The third supplementary solution, designated C13, supplied thirteen elements: aluminum, arsenic, cadmium, strontium, mercury, lead, lithium, rubidium, bromine, iodine, fluorine, selenium, and beryllium. Sodium and chlorine, though not singled out, were provided from several sources in these solutions.

In experiments with lettuce and asparagus a marked improvement in growth was observed from supplying, in addition to A4, the B7 solution. The further addition of thirteen more elements supplied by the C13 solution produced no measurable effect on either the lettuce or the asparagus plants. The results obtained with lettuce are given in Table I.

TABLE I

Effect of Adding Different Groups of Micronutrients on the Growth of Lettuce Plants in Culture Solution (from Arnon, 2)
(Average fresh weights in grams)

Micronutrients Added	Shoots	Roots
None	71.4	14.5
A4 (B, Zn, Cu, Mn)	105.7	22.0
A4 + B7 (Mo, Ti, V, Cr, W, Co, Ni)	1068.3	188.6
A4 + B7 + C13 (Al, As, Cd, Sr, Hg, Pb, Li, Rb, Br, I, F, Se, Be)	984.4	196.2

The results indicated that one or more of the seven elements contained in the B7 solution is capable of markedly benefiting plant growth. The question arose what significance is to be attached to an increase in growth in evaluating the essentiality of an element in plant nutrition. Mention was already made of the frequent "beneficial" effects from various soil treatments, and that under special conditions of nitrogen nutrition, molybdenum, chromium, and nickel were associated with favorable responses on the growth of barley. It was undertaken, therefore, to formulate definite criteria of essentiality by means of which the status of each of the seven elements comprising the B7 group could be tested.

The following criteria were set up (*9*): an element is not considered essential unless (*a*) a deficiency of it makes it impossible for the plant to complete the vegetative or reproductive stage of its life cycle; (*b*) such deficiency is specific to the element in question and can be prevented or corrected only by supplying this element; and (*c*) the element is directly involved in the nutrition of the plant quite apart from its possible effects in correcting some unfavorable microbiological or chemical condition of the soil or other culture medium.

The criterion of the foremost physiological significance is the requirement of an inorganic element for the successful completion of the life cycle of a plant. This is, of course, different from merely demonstrating a favorable effect on growth. The experimental procedure involved in putting this criterion to the test must be based on removing the element in question from the nutrient medium of the plant. This, however, is beset with difficulties; first, it is impossible to remove *completely* an element that may be contained in the seed. Second, the same obstacle applies to the nutrient medium. Regardless of how effective purification procedures are, they cannot be regarded as having removed the last atom of a contaminant originally present in the water and nutrient salts, or one that is derived from the container in which the plants are grown, or one gaining access to the nutrient medium in the course of an experiment.

Experimentally the problem resolves itself into selecting a species which has a high requirement for a given micronutrient and using purification procedures capable of reducing to a minimum the level of contamination in the nutrient medium. Different species vary greatly in their requirement for a given micronutrient. Beans, for example, have a far greater requirement for boron than barley. Alfalfa is capable of absorbing enough zinc from a medium in which corn shows acute deficiency symptoms. The extent to which it is necessary to purify the culture medium in order to produce deficiency symptoms may be reduced through selection of plants having a high requirement for an element.

In our experiments the water-culture technique was used and the tomato was selected as the principal test plant. This plant is characterized by a relatively small seed in relation to the emerged plant, thus rendering it likely that seed reserves would prove inadequate for the requirements of the growing plant which is, in addition, capable of successive vegetative and fruiting cycles. The indeterminate type of growth is desirable when an experimentally produced deficiency is later to be corrected by adding the missing element.

In purification of the nutrient medium, stress was laid first on the preparation of nutrient media of reproducible degree of purity and, second, on determining and expressing the level of remaining contamination in quantitative terms (*49*). It was possible, using this technique, to obtain consistent and reproducible responses in plants by adding minute amounts of metals to the nutrient medium (for example, 1 part of zinc in 200,000,000 parts of culture solution, which amounted to 0.001 mg. of zinc to a plant).

The proof that molybdenum is an essential nutrient for higher plants illustrates how this approach was followed in practice. When tomato plants were grown from the seedling stage in nutrient media purified in this manner and supplied with a complete nutrient solution including the four micronutrients, boron, manganese, zinc, and copper, characteristic deficiency symptoms became apparent in a few weeks (*10*). The lower leaves developed a distinct mottling, different from any other deficiency symptom previously noted in the tomato. In later stages, necrosis at the margins and a characteristic involution of the laminae accompanied by abscission of blossoms were noted. Thus, it was found that by the first criterion of essentiality, the completion of the life cycle, the nutrient medium was deficient in some essential element. Complete recovery was obtained upon adding the B7 solution. A breakdown of this group of seven elements disclosed that molybdenum was the needed micronutrient. The development of these deficiency symptoms was prevented by adding 1 part of molybdenum as molybdic acid to 100,000,000 parts of nutrient solution.

After experimentally producing a characteristic deficiency syndrome and demonstrating that it could be prevented or cured by the addition of molybdenum, the next step was to show that this effect was peculiar to molybdenum and that other elements could not be substituted. This was done by supplying the cultures with the six other metals in the B7 solution and with the thirteen elements in the C13 group. The deficiency symptoms persisted unless molybdenum was provided. Neither was there any additional improvement in growth when the application of molybdenum to molybdenum-deficient cultures was accompanied by the other elements in the B7 and C13 solutions.

To test the results by the last criterion, that of the direct effect of an element on the plant as distinguished from its possible influence on the root environment, molybdenum-deficient plants were sprayed with a dilute solution of molybdic acid (1 p.p.m. molybdenum) so as to bring about absorption only through the aerial parts of the plant. Recovery and resumption of normal growth with the disappearance of the molybdenum-deficiency symptoms took place. This provided the last link in the chain of evidence for the indispensability of molybdenum for the tomato plant.

The results with the tomato plant were subsequently repeated with mustard and lettuce. The essentiality of molybdenum was soon confirmed by Piper (*39*), Hoagland (*21*), and more recently by Hewitt and Jones (*19*) and Mulder (*38*). Molybdenum was recognized as an essential element and included with the A4 solution which was redesignated A5, whereas the B7 was changed to B6. The addition of A5 to a basic nutrient solution gave for the species tested the same results as a further addition of B6 and C13.

Does the inclusion of molybdenum among the essential elements complete their list? An unequivocal answer to this question cannot be given, notwithstanding the fact that a number of different species of plants have been grown successfully in rigidly purified nutrient solutions which supplied only boron, manganese, copper, zinc, and molybdenum. It is certain that, despite all caution, minute impurities of other elements persisted in the nutrient medium as well as in the seed. It would seem best to attempt to answer this question in a quantitative rather than a qualitative manner: to determine analytically whenever possible the upper limit of impurity for a given element that may be contained in the nutrient medium and to measure a growth response with and without a further addition of the element in question. This can be illustrated as follows. It was found with the dithizone test that when the combined zinc, copper, lead, cadmium, and mercury content of a nutrient solution was less than 0.0001 mg. per plant, severe deficiency symptoms occurred in the tomato. Recovery was brought about by adding 0.002 mg. of copper and 0.002 mg. of zinc, but no further improvement was produced by supplying 0.0005 mg. each of lead, cadmium, and mercury. These results, while confirming the indispensability of zinc and copper in amounts greater than those found in the nutrient medium, were interpreted as permitting no final conclusion as to the role of cadmium, lead, and mercury. The possibility that these elements, or others studied by a similar technique, may be required in amounts smaller than the incidental impurities which could not be removed from culture solution by the present technique cannot be a priori excluded.

If these views are accepted there can be no objection to regarding almost every element in the periodic table, and particularly those most frequently encountered in plant tissues, as susceptible of being shown at some time to be essential for plants. What can be asserted definitely is that, if an element now regarded as dispensable for a given plant should at some future time be found essential, it will be shown to be required in exceedingly small amounts—within the limits of contamination still encompassed by the refined methods now used for purifying the nutrient medium. This quantitative approach to the problem of essentiality of micronutrients is regarded not as a mere theoretical generalization but as a point of view conducive to a search for more refined analytical methods and procedures for growing plants which would make it possible to investigate the status of a number of new elements in plant nutrition.

The discussion thus far has dealt with those advances in the exploration of essentiality which were made through experimental modification of the external medium. Failure of the plant to grow was taken as the physiological yardstick by which nutrient requirements were measured. Except as a general surmise, experiments of this kind do not help to determine the function which the essential element plays within the plant. Regardless of how many different functions an element may perform within the plant, it is obvious that if the insufficiency of a nutrient resulted in blocking only one crucial reaction, growth would be arrested.

These considerations suggest an alternative approach to the problem of essentiality of inorganic nutrients. Rather than measuring the effect of the removal of an element from the external nutrient medium on growth, would it not be possible to indentify either an essential cellular constituent or a crucial biochemical reaction in which the inorganic element participates? For some of the essential elements the answer to this question has been obvious as soon as the chemical constitution of cellular substances was established. There was no difficulty in assigning an indispensable role to carbon, hydrogen, and oxygen solely on the basis of their entering into the composition of all living matter. As for the elements derived from the soil, the indispensability of nitrogen, sulfur, and phosphorus was adduced at an early period from their identification with proteins and nucleoproteins. As far as cations are concerned, the discovery that magnesium is an integral part of the tetrapyrrolic chlorophyll molecule assured an essential status to that element irrespective of what other functions it may perform in the plant. Calcium combines with pectic acid to form calcium pectate in the middle lamella of the cell wall.

This leaves only two elements on the classical list, potassium and iron, to which no essential status was assigned solely on the basis of their entering into the chemical composition of cellular constituents. With respect to potassium this state of affairs persists to this day. No organic compounds containing this essential element have been detected among the components of plant cells although suggestions have been made that it may combine with proteins. The case of iron deserves special treatment. Although it is known today to be an essential component of cellular constituents, that discovery followed an initial path rather distinct from analytical biochemistry. It resulted from a series of investigations whose primary objective was the understanding of a physiological process. In the case of iron the process investigated was respiration.

Before embarking on this phase of our discussion, it might be well to state certain premises. A convincing demonstration that a given element is indispensable to some vital process would suffice to establish its essentiality, even in the absence of appropriate growth experiments or corroborative analytical evidence on plant constituents. It is not inconceivable that for some micronutrient required in exceedingly minute quantity, the previously discussed experimental difficulties would make it impossible to remove completely the element in question either from the nutrient medium or the seed. On the analytical side, the element may enter into the composition of some organic intermediate of such great structural instability or low concentration in the tissue, or both, as to escape detection.

It is interesting to note that an approach to essentiality through the study of function was initiated over a half a century ago, in a manner which proved of historic importance to general biochemistry. In 1897 Bertrand (*15*) in France, reported that manganese was consistently associated with the activity of an oxidizing enzyme in plants, laccase. He came to regard manganese as an essential constituent of the oxidase system and hence essential to plant life. This announcement linked for the first time a metal with an oxidizing enzyme in living cells. It is true that recent work has shown that the effective metal in laccase is copper rather than manganese (*31, 51*), but the principle first proposed by Bertrand has retained its force.

The most fruitful development of the functional approach to essentiality of micronutrient had to await the evolution of modern biochemical techniques. The small amounts in which these nutrients were required by plants pointed very early to their probable catalytic function and, as first suggested by Bertrand (*15*), their association with enzymes. But the experimental proof for this hypothesis came much later. For boron, manganese, and molybdenum, we lack to this day precise formulation of their biochemical function in the plant. Their indispensability was established by growth experiments. Even for the others to which recent research has assigned some biochemical function, their essential status was already known from growth experiments. Thus the history of the micronutrients reveals that important physiological advances and the agricultural application of our knowledge of the indispensability of iron, boron, manganese, copper, zinc, and molybdenum occurred either in advance, or in the absence, of any knowledge of their functions within the plant.

The biochemical approach, however, has led to important advances in recent years. The study of respiration led Keilin (*28*) and Keilin and Hartree (*29*) to the identification of four iron porphyrin compounds which constitute the cytochrome system and are essential in the respiration of aerobic organisms. Here then was biochemical evidence of such validity that it would have established the essential status of iron as a nutrient, even if results of growth experiments were not available. Two other enzymes found in plants, peroxidase and catalase, have as their prosthetic groups iron-porphyrin compounds. Another enzyme widely distributed in plants and capable of participating in the respiratory process is polyphenoloxidase. This enzyme depends for its activity on the reversible oxidation and reduction of copper. Copper is the prosthetic group of the enzyme and cannot be replaced by any other metal (*33, 30*). Other copper enzymes in plants are laccase, noted previously, and ascorbic acid oxidase. In recent years the enzyme carbonic anhydrase was isolated from red blood corpuscles and was shown by Keilin and Mann (*32*) to have zinc as a prosthetic group. Present indications are that carbonic anhydrase also occurs in higher plants (*16*).

In the examples just cited the metal micronutrient is the prosthetic group of the enzyme. Its place cannot be taken by any other element. In this it fully meets the test of specificity which has been previously designated as a criterion of essentiality in growth experiments. It was already implied, however, that the identification of an element with a specific function in no way excludes other roles which the element may perform. An excellent illustration of this principle is the well-established property of many divalent ions to serve as activators of enzyme systems. The first stage in the metabolic transformation of hexose is the transfer to it of a phosphate ester group from adenosinetriphosphate. This is mediated by the enzyme hexokinase, which has been isolated from yeast. The enzyme is inactive in the absence of magnesium, and a relatively high concentration of this ion is required for full activity, (*14*). Arginase from both plant and animal tissues is activated by addition of Mn^{++}, Co^{++}, or Fe^{++}; of these Mn^{++} is the most effective (*18*). The carboxylase of *Proteus vulgaris* which catalyzes the oxidative decarboxylation of pyruvic acid to acetic acid and carbon dioxide is activated by the addition of Mn^{++}, Mg^{++}, Fe^{++}, Co^{++}, Ni^{++}, and Zn^{++} (*50*). Of special interest is the manganese activation of β-carboxylases since these enzymes catalyze reactions causing assimilation of carbon dioxide and leading to the formation of di- and tricarboxylic acids of importance in intermediary metabolism (*46*).

Space will not permit the mention of many other instances of enzyme activation by metals which have come to light in recent years. Several general conclusions, however, seem apparent. In some cases the activation appears to be specific for one element or at least it is greatly more efficient with it than with any other. This was shown to be the case for magnesium and manganese. Since both of these elements are known to be essential for growth, the evidence of their importance in specific enzyme reactions can be taken as an elucidation of their metabolic function. In instances where several cations activate a given system the evidence does not permit an unequivocal decision as to which element is the actual activating agent in vivo. Nor indeed is there any compelling reason to assume a priori that in the living cell, reactions of this kind must always be catalyzed by one element only. The situation becomes even more complex when work with isolated enzymatic systems brings to light the activating properties of elements such as nickel which are not known to be essential for life.

It is possible that for certain cellular reactions specificity of the activator is not a biological requirement. If this view is to be accepted, how can it be reconciled with the well-established specificity of each of the essential elements in growth experiments? It may be assumed that among the several functions which an essential element performs there is at least one for which it is specifically required, no substitution being possible. This view would retain the concept of essentiality in the sense that no sum of partial substitutions for individual reactions, assuming that they all became known, could ever succeed in replacing an essential element. It would, of course, be in accord with the known facts for most of the essential elements. Magnesium, for example, acts as a nonspecific activator of certain enzyme systems though it is also a specific activator for others and no other element can take its place as a component of chlorophyll. This hypothesis would also be compatible with reports of "beneficial" effects on the plant from the addition of nonessential elements. Sodium, for instance, could partly substitute for potassium in the sense that it could take over, at least in part, one of its functions. Beneficial effects from adding nonessential elements would thus merely reflect suboptimal conditions with respect to the supply of the essential elements. According to this view a nutrient medium possessing an optimum supply and favorable conditions for the absorption of the essential elements cannot be improved by the addition of nonessential elements, subject of course, to such reservations about possible unknown micronutrients as were discussed earlier.

The discussion thus far has tended to show how, in many instances, the study of function has strengthened or clarified the conclusions derived from growth experiments about the indispensability of inorganic nutrients. Recent studies in our laboratory have also shown, however, that conclusions drawn from growth experiments can greatly aid in the interpretation of biochemical observations on function. Because of the pertinence that these results (*12*) have to the problems under discussion, it is proposed to relate them in some detail.

It should be stated at the outset that the objective of the investigation which yielded the results to be examined was somewhat different from the topic under discussion. For the past several years we have been interested in exploring the possible function that inorganic elements, already recognized as essential for plant growth, may have in photosynthesis. We were encouraged to embark on this investigation by the important discovery of Hill (*20*) that the long-known capacity of isolated chloroplasts to evolve oxygen can be greatly enhanced by the use of suitable oxidants. Here was a subcellular system which retained the ability to carry out in vitro the photochemical reaction peculiar to the photosynthesis of green plants: the evolution of oxygen resulting from the splitting of water through the capture of the energy of light. The evidence in favor of the identity of the oxygen-liberating mechanism in isolated chloroplasts with that in the intact green cells has recently been reviewed by Holt and French (*24*) and further elaborated by Arnon and Whatley (*11*).

The photochemical evolution of oxygen by chloroplasts isolated from sugar beet and spinach was recently investigated by Warburg and Lüttgens (52) who reached the rather striking conclusion that the chloride ion was a coenzyme essential for the photochemical reactions in photosynthesis. That such a simple yet important fact escaped the notice of all the workers in this field was indeed cause enough for Warburg and Lüttgens (52) to remark how rash were all the previous theories on the mechanism of photosynthesis. The evidence which led these authors to conclude that chloride is a coenzyme of photosynthesis was as follows. The isolated chloroplasts lose their capacity for oxygen evolution after several washings in water. They can be reactivated, however, by adding the cytoplasmic fluid. The factor in the cytoplasmic fluid responsible for the reactivation of the chloroplast was found to be heat-stable. An analysis disclosed that the cytoplasmic fluid contained chloride in 0.08 molar concentration. The addition of chloride alone as $M/150$ potassium chloride brought about complete reactivation. Of the other anions tried, bromide was almost as effective, iodide and nitrate much less so, and fluoride, sulfate, thiocyanate, phosphate, and all the cations tried were without effect. Since chloride was the effective anion found in sufficient concentration in the cytoplasmic fluid, Warburg and Lüttgens concluded that it was the natural coenzyme of photosynthesis.

Impressive as this chain of biochemical evidence was in support of chloride as a coenzyme of photosynthesis, it posed at once a rather perplexing physiological problem from the standpoint of plant nutrition. Chloride is not generally regarded as an essential element for the growth of higher plants. Is it then possible that plants can get along in nutrient solutions without a coenzyme required for photosynthesis, a process indispensable for growth? The fact that Warburg and Lüttgens found appreciable amounts of chloride in their plants was not surprising. Chloride is widely distributed in soils and readily absorbed by most plants. Its presence in the plant, however, was hitherto regarded as incidental.

We undertook to investigate the problem by growing sugar beet and chard, in nutrient solutions *without* chloride.* As was expected the plants made excellent growth in the nutrient solution to which no chloride was added. The chloroplasts from these plants were isolated (*4*) and their oxygen evolution under the influence of light was measured manometrically, by a technique (*11*) similar to that used by Warburg and Lüttgens.

Our results disclosed important areas of agreement with those of Warburg and Lüttgens as well as several differences. An analysis of

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*These data have been published separately: D. I. Arnon, and F. R. Whatley, *Science,* 110:554 (1949).

both the chloroplasts and the cytoplasmic fluid showed no chloride in either, as would be expected in plants grown without chloride. The chloroplasts, even without washing, showed only feeble oxygen evolution. Unlike the experience of Warburg and Lüttgens, the addition of the cytoplasmic fluid failed to reactivate them, but it was already noted that our cytoplasmic fluid contained no chloride. On the other hand, we fully substantiated the finding of Warburg and Lüttgens that the addition of chloride brought about the activation of chloroplasts, giving us stoichiometric yields of oxygen in relation to the oxidant used. The effect of chloride on the course of oxygen evolution by illuminated chloroplasts is shown in Figure 1. We also confirmed the findings of these authors with regard to the influence of other anions on oxygen evolution (Figure 1). Bromide had an activating effect about equal with chloride; nitrate and iodide were much less effective; and sulfate, phosphate, thiocyanate, and acetate were without effect.

How should these results be interpreted? The intact plant is able to carry on normal photosynthesis without chloride, as judged by its excellent growth despite the absence of this ion either in the nutrient medium or in the leaf tissue. Yet when chloroplasts are isolated from the same plant, they require chloride for the vigorous progress of the photochemical reaction. One explanation would be that chloride acts in the leaf as a micronutrient and that minute amounts of chloride which would escape detection by the usual chemical analysis are nevertheless present in the nutrient medium as an impurity and find their way to the leaf. This explanation, however, although it cannot be ruled out entirely, is rendered unlikely by the data presented in Figure 2. In this chart the *rate* of oxygen evolution by illuminated chloroplasts ($Q_{O_2}^{chl}$) is plotted against chloride concentration. It will be seen that, whereas small additions of chloride brought about appreciable activation, a fairly high concentration, around 0.007 M, is required for full activation. This is in agreement with the value of $M/150$ potassium chloride reported by Warburg and Lüttgens (52) as necessary for full activation in their experiments. Such relatively high concentrations of chloride are not uncommon in soil-grown plants, but there is strong evidence from these and numerous other experiments that plants can make excellent growth without the presence of measurable amounts of

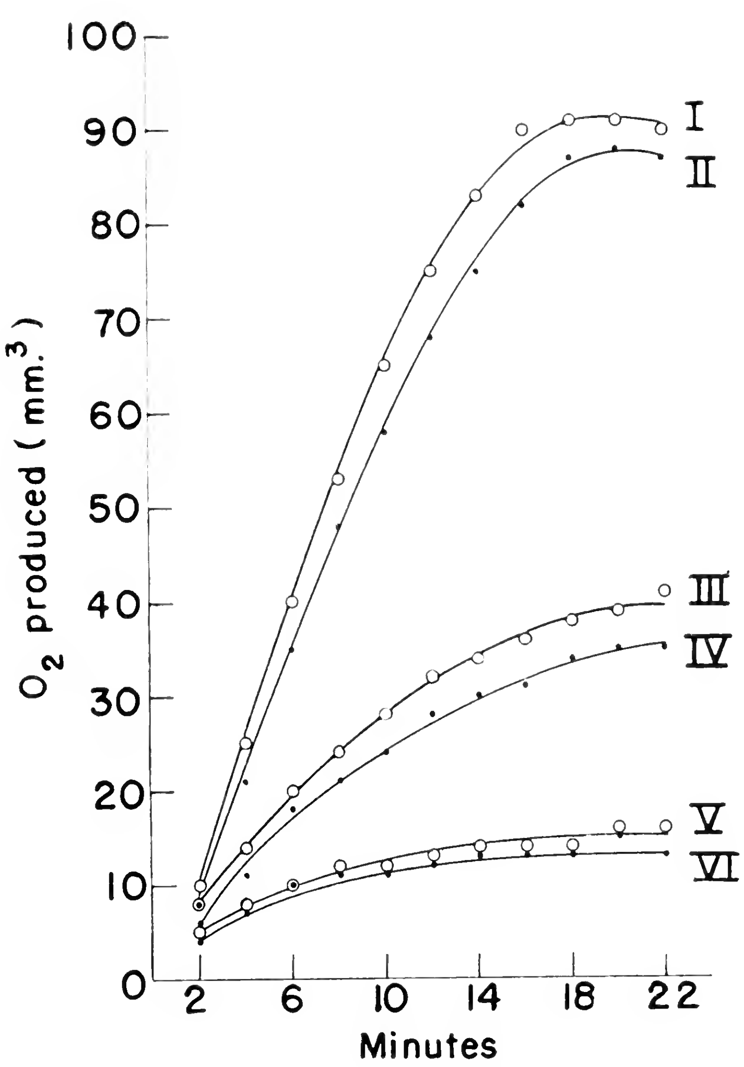

FIGURE 1. Effect of anions on oxygen evolution by illuminated chard chloroplast fragments. Curve I, effect of Cl^-; Curve II, effect of Br^-; Curve III, effect of NO_3^-; Curve IV, effect of I^-; Curve V, NaF; Curve VI, control. Values for sulfate, thiocyanate, and acetate coincided with those for the control. A potassium salt of each respective anion was added to give a concentration of $10^{-2}M$ in the manometer vessel, except that the fluoride was added as NaF. Reaction mixture: a chloroplast suspension containing 0.5 mg. of chlorophyll, $M/15$ phosphate buffer, quinone as oxidant. Illumination at flask level approx. 28,000 lux. t = 15° C. Other details of technique were similar to those previously described.—Arnon and Whatley (*11*).

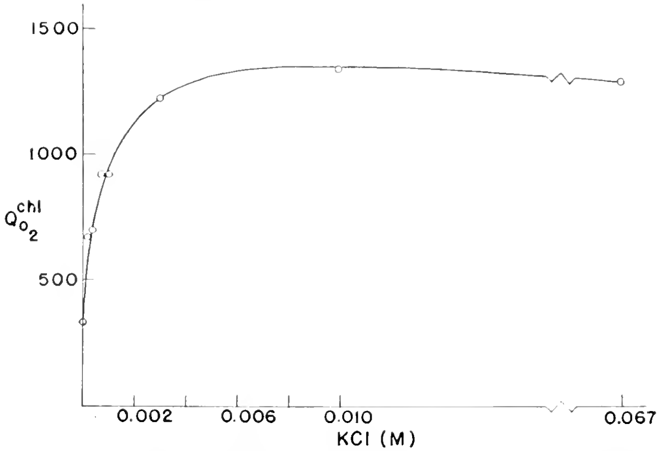

FIGURE 2. Effect of KCl concentration on rate of oxygen evolution by illuminated chloroplast fragments. $Q_{O_2}^{chl}$ = cubic millimeters of oxygen, per hour, per milligram of chlorophyll, computed from data obtained for the six-minute period from 1 min. to 7 min. after turning on the light. t = 20° C. Conditions not specified were similar to those given in the legend for Figure 1.

chloride either in the nutrient medium or in the plant. The other anion capable of giving full activation of photochemical oxygen evolution, bromide, although it is readily absorbed and tolerated by plants in appreciable amounts, is not a common constituent of plants or soils, and there is even less reason for suspecting it as being essential for plant growth.

If the view that chloride or bromide is a coenzyme of photosynthesis in vivo is to be abandoned, how can the effect of these anions in vitro be explained? We have formulated the hypothesis that, while in the intact green cell photosynthesis goes on without the participation of either chloride or bromide, once the cell is broken, there is a rapid light-induced deterioration of some cellular substance essential for the photochemical evolution of oxygen by chloroplasts. Chloride or bromide is able to protect this substance against inactivation, but the intact cell accomplishes this in some other manner. This would explain the superfluousness of the halide in the in vivo system as contrasted with its requirement in the in vitro system.

The hypothesis was tested in the following manner. Isolated chloroplast fragments were illuminated, without, however, adding the oxidant (in this case ferricyanide) which is necessary for the evolution of oxygen to take place. In one instance, chloride was added to the illuminated chloroplasts; the control contained no chloride. After twenty minutes of pre-exposure to light, the oxidant was added and the photochemical oxygen evolution was measured manometrically. To the

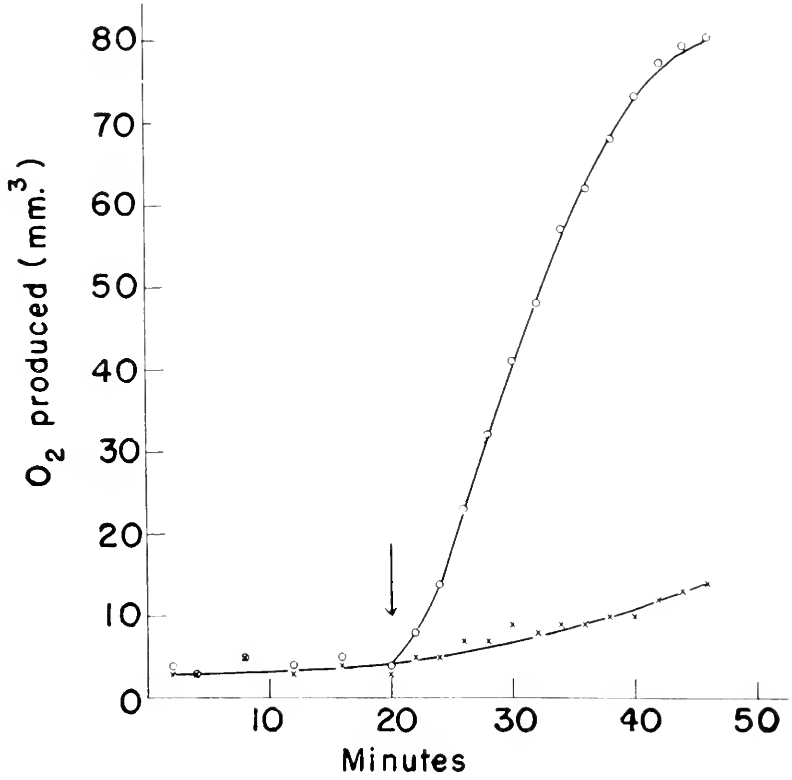

FIGURE 3. Protective effect of chloride on illuminated sugar beet chloroplast fragments. *Circles:* Illuminated for 20 min. in the presence of chloride. At t = 20 min. tipped in the oxidant (ferricyanide) to the manometer vessel. *Crosses:* illuminated for 20 min. in the absence of chloride. At t = 20 min. chloride and ferricyanide were added simultaneously. The concentration of chloride was 0.01 *M* KCl. 1.5 × 10^{-7} moles of $K_3Fe(CN)_6$ was added to each vessel. Conditions not specified were the same as those given in the legend for Figure 1.

chloroplast suspension which was exposed to light in the absence of chloride, this anion was added simultaneously with the oxidant. The results are shown in Figure 3. The pre-exposure to light in the absence of chloride inactivated the oxygen evolution system of the chloroplasts. This inactivation was irreversible. The subsequent addition of chloride had only a slight reactivating effect. On the other hand, a vigorous oxygen evolution giving stoichiometric yields resulted from the chloroplasts which had received added chloride during their exposure to light. Thus, chloride appeared to exert protective action on some essential photosynthetic factor that in the absence of this anion was irreversibly destroyed by light. Chloride also seemed to exert some protective action on the chloroplasts in the dark. There was evidence of inactivation from shaking the chloroplasts in the manometer vessels at 15° C. for a period equal to the light exposure. The inactivation in light, however, was much more pronounced. It goes without saying that the identification of this substance would be of great physiological interest. Experiments along this line have been under way in our laboratory, but no statement is possible at this time.

In addition to chloride, Warburg and Lüttgens (52) reached a conclusion of great significance with regard to zinc. They considered that the photochemical evolution of oxygen by chloroplasts is catalyzed by a metal and that in all probability the metal concerned is zinc. The evidence for this conclusion was as follows. The oxygen evolution reaction was strongly inhibited by *o*-phenanthroline, a well-known metal complex former; this reagent forms complexes with bivalent iron, nickel, cobalt, and zinc. The *o*-phenanthroline inhibition was reversed and the chloroplast fully reactivated by adding an excess of zinc. (In the example cited by Warburg and Lüttgens, ten times as much as would be required to bind the *o*-phenanthroline.) The addition of iron brought only partial reactivation. If, however, the metal was added to *o*-phenanthroline prior to placing it in contact with the chloroplasts, then zinc was not the only element which prevented inhibition: iron was equally effective, as were divalent cobalt and nickel ions. Warburg and Lüttgens (52) reasoned that since *o*-phenanthroline inhibition was observed only when this reagent was still free to combine with the metals in the chloroplasts, it is probable that some metal is involved as a catalyst in the oxygen evolution. They analyzed for the inorganic constituents of chloroplasts including iron and zinc and, on the basis of the amount of zinc found and the amount of inhibitor added, concluded that zinc was the metal concerned in the oxygen evolution reaction of chloroplasts.

The problem of *o*-phenanthroline inhibition of the oxygen-evolution reaction has been independently investigated in our laboratory as part of a general study of chloroplast reactions (*12*). In agreement with Warburg and Lüttgens, we found that *o*-phenanthroline inhibition was fully reversed by an excess of zinc and much less effectively by iron. Zinc was also very effective in reversing the inhibition even when added not in a tenfold excess but in a stoichiometric amount in relation to *o*-phenanthroline (Figure 4). However, when added in stoichiometric amounts, two other metals not known to be essential for plant growth, nickel and cobalt, were found to be even more effective in reversing *o*-phenanthroline inhibition. In our experience these two metals not only protected by binding the *o*-phenanthroline before it was mixed with the chloroplasts as observed by Warburg and Lüttgens (52), but also reactivated the previously inhibited preparations in a manner similar to zinc (Figure 4).

Reactivation was also obtained with copper, a micronutrient of established status, known to occur in chloroplasts. The reactivating efficiency of copper was, on the basis of stoichiometric amounts, intermediate between iron and zinc. Unlike iron,* however, an increase in the concentration of copper gave total reactivation in a manner similar to zinc. As seen in Figure 4, doubling the concentration of copper brought about a marked increase in its effectiveness.

It was found then that the reversal of *o*-phenanthroline inhibition was accomplished by two elements, nickel and cobalt, not known to be essential for plant life and by two, copper and zinc, recognized as micronutrients. It is considered unwarranted, on the basis of evidence now available, to associate any one metal with the photochemical evolution of oxygen. Deductions on the basis of composition of plant tissues appear to be, in the light of previous discussion, not wholly reliable.

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*After this manuscript was submitted for publication, new evidence on this point became available. It will be published elsewhere.

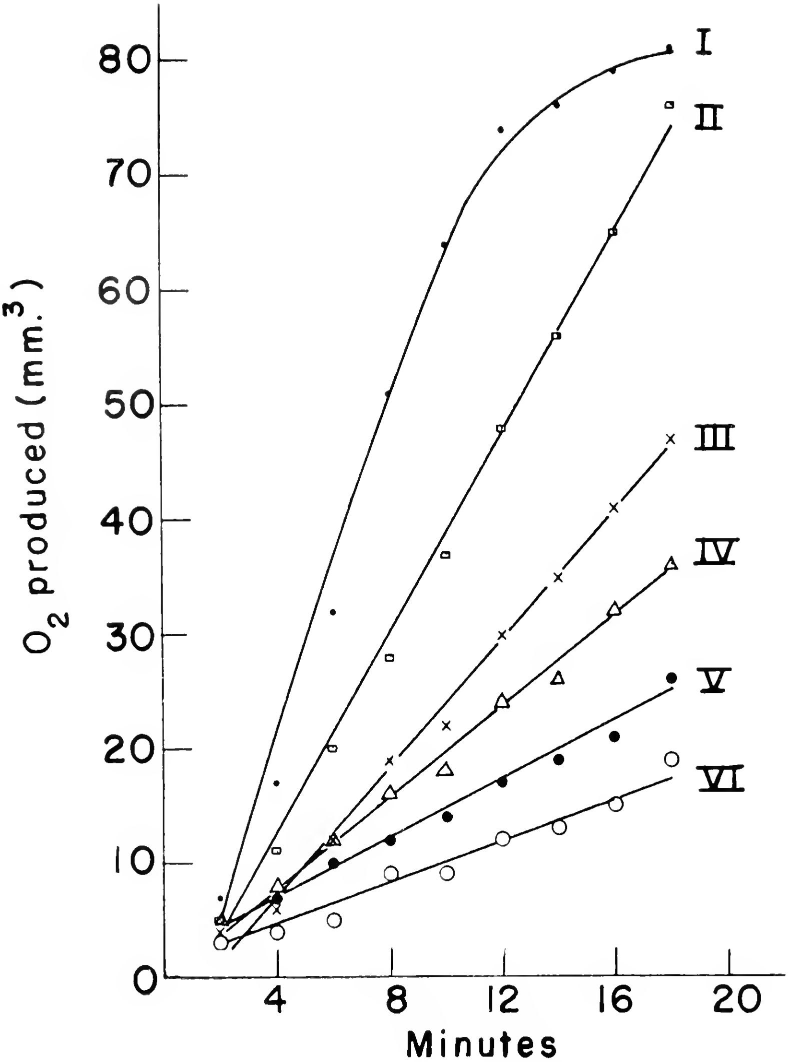

FIGURE 4. Effect of metals on reversal of *o*-phenanthroline (*o*-P) inhibition of sugar beet chloroplasts. *o*-Phenanthroline was added to chloroplast fragments in all cases except to the control represented by Curve I. Curve II, effect of adding nickel and cobalt and doubling the copper concentration; Curve III, effect of zinc; Curve IV, effect of copper; Curve V, effect of iron (ferrous); Curve VI, *o*-phenanthroline alone. The addition of chromate was without effect. The concentration of *o*-P was $5 \times 10^{-5} M$. The metals were added as sulfates and nitrates in a $5/3 \times 10^{-5} M$ concentration to give a stoichiometric ratio of 3 *o*-P: 1 metal. Conditions not specified were the same as those given in legend for Figure 1.

This it not to say that zinc is not the metal specifically concerned in the photochemical reactions of photosynthesis, but to suggest that such a conclusion is as yet not supported by incontrovertible evidence. It is hoped that experiments now in progress may throw some more light on the subject.

The foregoing discussion has attempted to demonstrate that an integrated concept of the essentiality of mineral elements rests on the combined contributions from studies of inorganic requirements for growth and investigations on functional aspects of the essential nutrients. The two approaches, which for the sake of convenience may be designated as the physiological and biochemical, respectively, are mutually supplementary. It was shown how in the case of chloride, its proposed essential status as a coenzyme for photosynthesis could not be accepted in the light of evidence from growth experiments. It is possible, however, that in the future an insight into the essential function of an inorganic element may be gained from biochemical studies, well in advance of any knowledge gained from growth experiments. A case in point is cobalt in animal nutrition. Cobalt was recognized as a micronutrient essential for ruminants (*36*), but there was no evidence that it was required by other animals. Recently a striking discovery was made that cobalt is a component of vitamin B_{12}, which is identified with the "antipernicious anemia" and the "animal-protein" factors (*42, 43, 41*). Vitamin B_{12} seems to be essential for all animals, and cobalt, which was not previously found in a compound of a natural origin, must therefore now also be regarded as an essential micronutrient for animals other than ruminants. (However, recent experiments indicate that in ruminants at least, cobalt may have functions distinct from its association with vitamin B_{12}; Becker *et al., 13*). Evidently the quantitative requirement for cobalt by nonruminants is so small that it escaped detection in direct growth experiments. An idea as to the experimental difficulties which may be involved is given by the fact that four tons of liver were required to yield one gram of the pure vitamin (*42*).

The study of the inorganic requirements of higher plants has, apart from its richly rewarding scientific purpose in contributing to the understanding of plant growth and metabolism, an important practical objective as well. Developments in this field have provided in the past, and will continue to provide in the future, a scientific basis for fertilization practices. As the list of essential elements has expanded, it was possible to answer with increasing assurance how many indispensable nutrients need to be supplied in the external medium to insure optimal plant growth. It is hoped that advances in our knowledge of the function of inorganic nutrients will provide a sound basis for estimating quantitative requirements of fertilizer elements for different crops at different stages of growth and in relation to climatic factors.

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CHAPTER

14

Mineral Nutrition in Relation to the Ontogeny of Plants

W. F. LOEHWING

The study of mineral nutrition in relation to ontogeny is merely one type of approach to an understanding and control of plants as organisms. A study of this sort becomes primarily an attempt to arrange the known facts of mineral metabolism as a progressive sequence of events as these relate to the commonly observed growth patterns of plants. The major problem is one of integration of somewhat isolated data with the growth process as a whole. It seems desirable to outline the accumulation and distribution of inorganic ions and then to trace as far as possible their connection with tissue differentiation and the progression of the entire life cycle of the plant as a whole.

An understanding of mineral nutrition in relation to ontogeny is complicated by the fact that numerous meristems give rise continuously to new tissues and organs, with the result that various parts of the plant are usually in different stages of development. Thus, comparable size and age of parts or of the plant as a whole are not necessarily criteria of developmental similarity (*121*). At mid-maturity of a plant, its various parts may display the entire range of development from youth to senescence (*11*). Upon these developmental differences between parts are superimposed certain ecological contrasts (*93*). During growth, a single plant often modifies its environment in such a way that organs appearing in a chronological sequence commonly form an ecological succession (*76*, *152*). Due to the progressive modification of apical meristems in the course of vegetative development, for example, there usually occurs an axial progression from mesomorphy of the lower leaves to comparative xeromorphy of upper leaves on the same stem (*76, 84*). It is also obvious that data on root physiology are essential to a comprehensive grasp of ontogeny. This fact explains the preference of investigators for liquid and gravel cultures from which roots are readily retrievable. Such cultures, in addition, permit better control and study of the substrate than in the case of soil-grown plants. Finally, a running inventory on the composition and behavior of the nutrient solution or substrate is a great aid to an understanding of the plant's relationship to and effect upon its edaphic environment (*1, 14, 26, 37, 57, 59, 60, 106*). Thus, Härtel (*52*) reports translocation of carbon dioxide from roots to shoots where it can be used in photosynthesis, and Ulrich (*135*) has noted the buffer action of organic acids in roots when cation absorption exceeds anion intake (*79*).

In scanning the numerous studies of developmental physiology, it is surprising to find many similarities in fundamental processes despite the extreme diversity of plant types and environmental conditions involved. As might be expected, a preponderance of data exists concerning herbaceous annuals, and the most consistent portion of this information relates to plants grown under controlled environmental conditions. Comparable data for biennials and perennials, often of necessity grown under field conditions, are more difficult to obtain, hence, often relatively incomplete and more difficult to correlate and interpret. Because of this fact the present discussion is limited to those aspects of developmental processes which seem common to the wide range of ordinary herbaceous annuals most frequently investigated.

THE VEGETATIVE STAGE

Under favorable growing conditions, the phase of rapid vegetative enlargement in typical annuals is characterized by progressive increments in absolute amounts of inorganic elements, carbohydrates, and proteins. Due to the accelerated synthesis of organic compounds, the proportion of ash on a percentage basis begins to fall even though absolute amounts of the latter may continue to rise until well toward maturity (*29, 71, 109*). Many annuals tend to absorb the major portion of their total mineral supply in very early life (*17, 77, 142*) and early absorption is, in general, in excess of current needs when the external supply is favorable. In early vegetative stages under conditions of balanced or constant supply, nitrogen, potassium, and phosphorus commonly increase faster than calcium, iron, magnesium, and sulfur due in part to the relative immobility of the latter elements within the plant (*3, 24, 71, 111, 112*). It may be noted, however, that the rate of absorption of a particular ion is determined not only by its availability in the substrate but also by the concentration thereof already in the plant (*20, 47, 71, 136, 151*). Numerous investigations (*11, 15*) indicate that the beneficial effects of soil fertilization are due primarily to increase in foliar area and of assimilative tissue rather than to increase in efficiency of assimilative processes.

As might be expected in early stages of development when new tissues are being formed on a large scale, the young plant is relatively more active in accumulation of water (*11, 55, 68, 99, 127, 140*), nitrogen, and protein than in its later life (*11, 54, 62, 75, 112, 138*). On the basis of large amounts of inorganic and amide nitrogen found in young plants under favorable conditions, much of the early intake is in the nature of luxury consumption (*47*). Experimentally, this is made evident by the fact that gradients of curves for nitrogen accumulation are usually steeper than those for dry weight gains in young plants (*11, 67, 75, 112, 138*). Recent work on growth substances and naturally occurring phytohormones indicates important interrelationships of such substances with nitrogen (*9, 10*), zinc (*126, 134*), and several other nutrient elements (*21, 81*). Auxin activity diminishes under conditions of nitrogen deficiency before lack of nitrogen causes retardation of growth or stem elongation (*133*). It has been observed that zinc controls tryptophane formation and, hence, auxin activity because tryptophane is an auxin precursor. Went (*141*) discusses the work of Bonner and others on the vitamin requirements of flax, pea, and tomato roots. Flax roots which require pyrimidine and thiazole for growth do not themselves synthesize these thiamin precursors but are normally dependent upon translocation of them from the shoot which can produce them. Root tissue-cultures of flax grow only when pyrimidine and thiazole are added. In this instance, sulfur functions as an organic complex in thiamin. Similar relationships seem to prevail in pea and tomato roots for nicotinic acid and vitamin B_6, respectively (*141*). These observations raise the question whether failure of nutrient absorption by roots when their carbohydrate supply is low, may not also be associated with failure of hormone translocation to roots from the shoot.

During the vegetative phase of growth, there is an intimate connection between carbohydrate and protein synthesis. Not only are carbohydrates and nitrogen used in the synthesis of proteins, but a portion of the soluble hexoses evidently provides the respiratory energy necessary for the chemical reduction of nitrates as an antecedent to amino acid and protein formation. In fact in young plants, the supply of soluble sugars appears largely to condition the rate of protein synthesis (*85, 86, 87, 127, 153*). Obviously, the availability of oxygen also is essential to the respiratory oxidation of a portion of the carbohydrates but this usually is not a limiting factor in early growth as it is later. Mothes (*85, 87*) has shown that all conditions, such as light, photosynthesis, and open stomata, which tend to raise internal oxygen tension, favor protein formation. Conversely, protein hydrolysis is accelerated by a low internal oxygen tension and by low water content in later development (*25*). Thus, the rate of photosynthesis as a source of both carbohydrates and oxygen is closely bound up with nitrate reduction and protein synthesis. During the later phases of active vegetative growth, the plant rapidly accumulates carbohydrates and appears to become relatively less efficient in protein than in carbohydrate elaboration (*11*) as shown by increments in the carbon–nitrogen ratio (*53*).

Thus from the period of germination to flowering, three fairly distinct stages of metabolism are evident. The conspicuous stages in the nutrition of the vegetative plant comprise an initial anabolic phase (I) in which intake of inorganic nutrients and synthesis of proteins is rapid. In the second phase (II), the accumulation of carbohydrates accelerates while the rate of protein synthesis gradually diminishes. As flowering is approached, a third or catabolic phase (III) becomes evident in which hydrolysis of reserves begins to overbalance synthesis and a general internal redistribution of nutrients is initiated. Though conditions of environment and nutrient supply determine to a considerable degree the exact time of the shift from predominantly anabolic to catabolic activity, the latter transition is characteristically associated with flowering and commonly initiated prior to anthesis (*40*).

THE FLOWERING STAGE

In reference to the initiation of reproductive processes, recent data (*13, 18, 80, 96, 144, 145, 150*) have confirmed and elaborated many older observations to the effect that the phenomenon of synapsis or sporogenesis represents a turning point in nutritional metabolism (55, 56, *61*, 65, *147*). (*a*) In monoecious species such as corn, it has been observed that the origin of staminate and pistillate organs is associated with a transitory but systemic acceleration of anabolic over catabolic processes including more rapid salt absorption and dry weight gains. (*b*) Under normal conditions the metabolic stimulus associated with synapsis is brief and soon gives way to a reduction in anabolic processes during the ensuing phase of blossoming or anthesis. The flowering phase is usually characterized by a subsidence of anabolic activity as well as the inauguration of fundamental modifications (*70*) and redistribution of organic and inorganic nutrient components (*11*, 25, *40*, *61*, *78*). Subsequent events vary considerably among species, some of which, for example, undergo no further elongation of the main axis following anthesis (*11*). While there are important differences between plants of determinate habit, such as grasses, and those of indeterminate habit, as in many dicots, the subsidence in rate of stem elongation can, nevertheless, often be taken as an index of the fact that reproduction is already under way even though gross morphological evidence of floral parts is not yet visible (*73*, *104*). (*c*) The decline of anabolic activity associated with blossoming gradually gives way to what is usually the final resurgence of absorption of mineral nutrients and acceleration of organic synthesis in vegetative tissues. This anabolic stimulus is associated with the fusion of male and female nuclei in syngamy and the very early enlargement of young fruits (*22, 38, 95, 96, 144, 145, 150*).

From the standpoint of ontogeny, much could be said in favor of beginning such a discussion with sexual fertilization or syngamy which, after all, is the actual inception of the new plant. Such a procedure would be justified not only by the chronological sequence of events but by the fact that many environmental factors to which the growing embryo is exposed prior to its development into a mature and dormant seed can predetermine in considerable degree the course of ontogeny subsequent to seed germination.

As stated, the onset of blossoming or anthesis is marked by an apparently simultaneous reduction in absorption by roots, an internal shift in water balance (*11, 37, 40, 56, 58, 63, 64, 107, 121, 122, 127*), and redistribution of both organic and inorganic nutrients (*28, 73, 89, 90, 127*). At the present time it seems that the foregoing phenomena are all the results of some as yet indefinitely defined regulatory or causative factors. We already have some evidence, however, that they are at least associated with, if not caused by, increments in growth substances at reproductive loci within the plant following syngamy (*144, 145, 150*).

During anthesis, the carbohydrate supply of roots and their rates of absorption commonly fall to low levels (*11, 25, 40, 43, 61, 78, 102, 137*). The inadequacy of root carbohydrates at this period has frequently been advanced as the cause of their low absorptive activity (*40*). As already noted, however, recent data indicate that the organic reserves of the root function jointly with growth substances in absorption and root enlargement.

In liquid and gravel culture experiments in which entire plants including roots and the nutrient solution have been analyzed, there is evidence of transitory excretion of certain mineral elements during anthesis (*4, 22, 38, 47, 51, 147*). Nitrogen and potassium especially often increase temporarily in the nutrient medium (*51*). The recent work of Cailachian (*23*) on nitrogen in relation to flowering indicates that there are three categories of plants—namely, those in which nitrogen accelerates, delays, or has no effect on flowering (*144*).

During anthesis, lower leaves show a rapid acceleration in loss of water (*82, 83, 121*) and organic reserves (*83*), a trend which subsequently reaches the extreme of protoplasmic disintegration and transfer of such cellular residues to reproductive organs and to younger vegetative tissues. In the early stages of this trend, loss of water is progressive despite appreciable increments in osmotic values of press sap. Hydrolysis of insoluble organic reserves of lower leaves is a conspicuous phenomenon at this time (*2*), yet the resulting rise in the osmotic pressure of tissue fluids is usually unable to arrest water loss (*11, 83, 103, 127*). The inference follows that factors other than osmotic pressures serve in regulation of the water balance of tissues (*83, 127*). Numerous studies on the drought resistance of plants have shown their extreme sensitivity to water shortages during flowering (*27, 68, 116, 129*). Even if plants survive drought at the flowering stage, they seldom show a normal course of development thereafter.

Smirnov (*127*) and his associates have shown by a series of ingenious experiments that water loss and hydrolysis of organic reserves occur simultaneously with a reduction in the amount of hydrophilic colloids in vegetative tissues. Smirnov stresses the "salting out" effect of mineral nutrients upon organic colloids and the resultant reduction in water retentivity by colloids after precipitation. Precipitation of cell colloids is evidently accompanied by liberation of previously absorbed enzymes and a simultaneous increase in their hydrolytic action. During this period, there also occur marked changes in pH of tissue fluids; such alterations may be additive to the effects of colloid precipitation in increasing the hydrolytic action of enzymes. Many investigations provide evidence of marked reduction in the rate of photosynthetic activity during the period of rapid hydrolysis of organic reserves (*11, 148*).

Relatively low levels of tissue moisture and hydrolysis of organic reserves in lower leaves frequently involve living protoplasm (*46, 49, 99, 108, 123, 124*), and, once the protoplasmic components of such tissues begin to undergo hydrolysis, they usually become incapable of renewed synthesis even upon amelioration of the water and nutrient supply (*83, 104, 132*). This observation is usually offered as the explanation of the early death and abscission of lower leaves of plants in the reproductive phase. Mothes (*87*) has shown the dependence of protein synthesis upon internal oxygen tension, and a reduction in rate of protein formation commensurate with the decline in the rate of photosynthesis. Smirnov's data (*127*), in turn, show a correlation between the rates of respiration and protein synthesis (*49*). It thus appears that photosynthetic oxygen favors protein formation by acceleration of aerobic oxidation of carbohydrates. The resultant energy is important to the reduction of nitrates as well as to the union of nitrogen with carbohydrate derivatives in amino acid synthesis. Smirnov points out that in the early life of annuals, protein synthesis parallels, and hence is presumably dependent upon, the concentration of hexose sugars. With the onset of reproduction, however, protein production ceases to be proportional to the soluble sugar content but parallels the rate of foliar respiration. Thus, in the vegetative phases, carbohydrate supply is the controlling factor in protein synthesis, while oxygen supply becomes a major regulatory factor in the synthesis of proteins during the reproductive phase.

In addition to the foregoing functional factors influencing the water economy of the flowering shoot, vascular tissues also undergo complicating structural modifications (*30, 31, 74, 113, 125, 130, 131, 143, 149*). The work of several investigators (*116, 149*) reveals a subsidence in cambial activity which begins in the vicinity of floral buds and commonly extends progressively toward the base of the stem (*125*). Phloem formation especially seems to be reduced and failure of vascular differentiation commonly involves pedicels or fruits stalks, thus often impairing fruit setting or fruit enlargement (*27*). The impairment of conduction appears during the flowering stage and retards the redistribution of nutrients; the reduction in conductive capacity of vascular bundles in stems and pedicels may become a temporarily limiting factor in the rate of growth of the shoot apex and of fruits.

THE FRUITING STAGE

The fruiting stage has its origin in syngamy. The early stages of fruit enlargement are commonly associated with marked increments in absorption by roots and accelerated anabolism of the younger parts of the shoot (*13, 18, 27, 34, 42, 65, 74, 91, 94, 100, 127, 144, 145, 150*). Gains in nitrogen and potassium become appreciably higher (*74*). Absorption of phosphorus and iron, though fairly steady at first, tend to rise, sometimes to surprisingly high levels as maturation supervenes (*44, 117, 139*). There is an increased accumulation of proteins and carbohydrates, the latter usually being the greater in terms of dry weight gains (*127*). The origin of the systemic stimulus to accelerated activity following syngamy appears to be associated with increments in growth substances at reproductive loci and their translocation to adjacent tissues (*8, 88*).

As would be expected, increasing amounts of organic and inorganic reserves are diverted from vegetative to reproductive organs as more fruits are set and their enlargement accelerates (*28, 73, 90, 145*). It is interesting to observe, however, the comparatively uniform composition of seeds and fruits in relation to the frequently great differences in mineral elements in the vegetative organs nourishing them (5, *105*). Seeds and fruits are highly selective in the elements which they accumulate from leaves and stems (5).

In recent years, agronomists studying the problems of fertilizer placement have reported significant differences in response to a given element with the stage of plant development at which it is supplied (*6, 7, 12, 16, 19, 32, 33, 39, 41, 45, 48, 50, 72, 97, 101, 105, 110, 114, 115, 118, 119, 120, 128*). Striking differential effects upon specific tissues and the course of ontogeny have been observed. There is evidence, for example, that nitrogen applications at or immediately following anthesis induce responses in reproductive and vegetative organs which are quite different from those observed in plants held continuously at uniform levels of nitrogen supply (*12, 33, 47, 101, 110, 114, 128*). During the early fruiting stage, many species exhibit a high absorptive capacity for and extreme sensitivity to increments or diminutions in nutrient supply (*34, 92, 114*). Their responses to fertilizers made available at this time are often wholly unlike those supplied at earlier or later stages (*132*).

Where marked deviations from ordinary growth patterns are observed following changes in nutrient level at mid-developmental stages, it becomes of interest to learn which tissues are primarily affected and in what manner, both as to function and structure. Detailed information on these responses is as yet quite meager, but judging from definite responses thus far reported, closer study of them should prove peculiarly productive in extending our understanding and control of fundamental features of plant development.

The recent work of Rankin (*110*) on staggered and late supplies of nitrogen in producing differential effects upon the number of spikes, florets per spike, weight and number of kernels per plant in wheat is a case in point (*12, 115, 120, 128*). Another is the work of Sybil (*132*) on tobacco in which a shift from low or medium to high nitrogen supply at anthesis produced a leaf structure and organization quite unlike those observed in plants grown at uniform nitrogen levels. It is also worth noting that the form in which nitrogen is supplied is important, as the effects of ammoniacal and nitrate nitrogen may be quite different (*154*). The yield of fruit produced by a given amount of vegetative tissue can be varied appreciably depending on the form of available nitrogen and aeration of the substrate (*146*). The effects of elements other than nitrogen also appear to vary significantly with the stage of development at which applied (*101, 105, 144*).

The phenomena of nutrient balance between vegetative organs and fruits, especially in heavily fruiting varieties such as cotton and tomato, have been described by many investigators. The effects of a heavy crop of fruit in depleting the nitrogen reserves of leaves and stems are rather well known, as also are the cyclic renewals of vegetative activity when fruit abscission or maturation occurs (*28, 73, 90, 137, 145*). The usual course of events in numerous annuals is the depletion of leaves to the point of protoplasmic disintegration and the rapid initiation of senescence terminating in abscission or death of leaves and excretion of mineral elements by roots to substrate (*35, 36, 37, 44, 61, 69*).

In conclusion it may be noted that, though mineral nutrients are not the initial and primary causes of tissue differentiation or inception of reproductive processes, they are often controlling factors in the implementation of the plants' developmental potentialities. Recent work discloses the intimate relation of mineral elements to the formative action of growth substances (*66*), to the respiratory action of nucleotides, and to other intermediates in oxidative metabolism. Thus, the action of phytohormones and other specifically morphogenetic compounds is intimately correlated with mineral nutrients much as the inorganic ions are related to photosynthesis, enzyme action, and other vital functions in the regulation of growth and the ontogenetic cycle.

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CHAPTER

15 Correlations between Protein-Carbohydrate Metabolism and Mineral Deficiencies in Plants

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INTERPRETATION of the data on protein and carbohydrate metabolism of the green plants subjected to mineral deficiency has been complicated by the use of a wide range of climatic conditions, differences in sampling, and the nonspecific nature of the methods of chemical analysis. Practically no information exists concerning the chemical mechanisms of symptom formation with mineral deficiency. The greatest difficulty, however, can be attributed to our meager information on the organic nutrition of the plant. It is for this reason that a brief introduction dealing with protein and carbohydrate metabolism is advisable before attempting to evaluate the effects of mineral deficiency.

PROTEIN AND CARBOHYDRATE METABOLISM

A diagrammatic outline of the probable course of organic nutrition has been published by Gregory and Sen (*14*) and is here reproduced in Figure 1. Chibnall (*6*) has pointed out how adequately it represents the facts as far as they are known.

An examination of the chemical reactions indicated in this diagram reveals that a breakdown of hexose molecules is assumed to precede the formation of organic acids. Interaction of these with ammonium ion results in production of amino acids. Protein is formed through condensation of amino acids and these are regenerated on subsequent hydrolysis. Amides may or may not participate in protein formation. Respiration, i.e., oxidation with liberation of carbon dioxide, is represented as having a multiple source, namely, breakdown of hexose, of aliphatic acids, and of amino acids.

The consensus of opinion hitherto has been that the protoplasmic proteins remained unchanged after formation, that is, only the storage proteins were labile. Recent studies by Vickery, Pucher, Schoenheimer, and Rittenhouse (*56*) disclosed that isotopic nitrogen supplied to the tobacco plant as ammonium salt appeared in its proteins within four hours. Calvin and Benson (5) could detect the presence of radiocarbon in the amino acids (alanine and serine) of an alga after a period of thirty seconds of photosythesis with C^*O_2. The extreme rapidity of these reactions is a striking demonstration of the manifold chemical reactions taking place in the hitherto presumably static protoplasmic proteins of the cell.

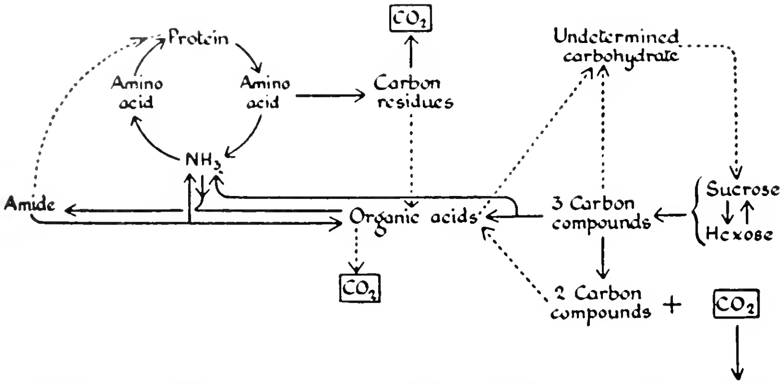

FIGURE 1. Diagram from Gregory and Sen (*14*) illustrating the probable course of protein and carbohydrate metabolism in plants.

This is one reason that the amino acid-protein cycle depicted in the above scheme seems to the writer to be the weakest part of the picture. Alternate condensation and liberation of amino acids would seem a rather futile procedure; especially so since there is some evidence that protein may participate in the formation of respiratory carbon dioxide. The writer is of the opinion that many of the metabolites of the cell, including other proteins and enzymes, are formed and liberated through cleavage from the permanent or protoplasmic proteins. Only the debris of these reactions would undergo complete oxidation to carbon dioxide and ammonia, or be reworked to carbohydrate or other compounds. This interpretation would be in better agreement with the surmise of Gregory and Sen that breakdown of protein follows a different course than synthesis.

Another objection to a simple regenerative amino acid-protein cycle in plants is based on the phenomenon of "nitrogen balance" in animals. A daily loss of amino nitrogen must be compensated in the diet, since it persists even during extreme starvation. The "wear and tear" protein metabolism is accompanied by loss of amino nitrogen as urea or uric acid, even in the starving animal. It seems logical to the writer, therefore, to assume that a similar cycle of nitrogen and carbon loss occurs in the plant. While traces of urea and uric acid have been identified in plants, respiratory carbon dioxide probably accounts for the carbon while the nitrogen is reutilized to form new amino acids. Vickery and others (*56*) have remarked on the close similarity between "nitrogen balance" in animals and the rapid intake by tobacco proteins of radionitrogen, though Vickery (*55*) categorically later denied the existence of "nitrogen balance" in plants. It is true that technically "nitrogen balance" as such does not exist in plants since nitrogen is retained and re-used. However, in the larger phenomenon of which it is only one facet, it must be assumed that loss of carbon and nitrogen from protoplasmic protein is inherent, automatic, and irreversible in all cells. It is truly cyclic.

Other data bearing on the cyclic interpretation of protein metabolism consist in the fluctuations of protein and carbohydrate in plants. Protein varies little in comparison to carbohydrate, if storage products of both are omitted from consideration. Brief starvation experiments readily demonstrate this difference. Protoplasmic protein can be depleted or altered to such an extent as to prevent resumption of growth only through starvation of long duration. This is not the case, within limits, with carbohydrate. Increases in carbohydrate above minimal have little influence on protein formation, whereas increases in nitrogen (particularly as ammonium salts) do and may lead to marked depletion of carbohydrate. It might also be mentioned that carbohydrate supplied externally to the green plant is utilized efficiently, whereas amino acids so supplied are usually harmful even in high dilution (*49*). Finally the so-called "sparing action" of sugar on respiratory destruction of amino acids (*27*) is in all probability a "replenishment action" instead. These and other data and interpretations will be found in reviews by Nightingale (*29, 30*), Chibnall (*6*), and Vickery (*54*). The writer has depended to a great extent on these and other workers for information of the accomplishments in the field of protein chemistry.

A brief reference might be made to the question of amino acid synthesis concerning which so little is known. It was possible by means of *Aspergillus niger* van Tiegh, a fungus, to demonstrate that normal and optimum growth could take place only with a mixture of glutamic acid, proline, and ornithine in the absence of sugar and other nitrogen (*46, 47*). The other amino acids, excepting aspartic, were ineffective, whereas malic acid was also usable. The aspartic and malic acids have the same carbon chain. Adoption of a classification of amino acids into three groups has received some measure of verification through the work of Steward and Street (*52*) and of MacVicar and Burris (*26*) on the importance of glutamic acid in amino acid synthesis. Stepka, Benson, and Calvin (*51*) found serine and alanine formed during photosynthesis to be radioactive, whereas the large quantities of glutamic acid elaborated were inactive. Synthesis of straight chain amino acids with 3 and 4 carbon atoms would seem to follow a different path than those with a 5 carbon atom chain according to their data.

THE DATA OF MINERAL DEFICIENCY

It is evident in view of the speed and complexity of nitrogen and carbon metabolism in plants that any deficiency of a mineral element participating directly in protein and carbohydrate reactions would be readily indicated through chemical analysis. The same would also be true for an indirect participation, as in enzymes controlling these reactions. However, deficiencies drastic enough to influence a basic reaction of protoplasm would affect all physiological processes and could not be accepted as proof of a role in a limited phase of nutrition.

A feature of mineral nutrition studies that has long puzzled the writer has been the chemical mechanism whereby characteristic deficiency symptoms are produced in the plant. Data obtained in aseptic culture with amino acids has indicated the probable participation of these compounds in these phenomena (*48, 49*). These data are included at the end of this paper.

Certain assumptions and simplifications have been introduced in the data of the literature here reproduced. All values have been recomputed in terms of "deficiency/control," i.e., content of a constituent in the deficient plants divided by that in the controls. Variations as between chemical methods so tend to be minimized. Carbon values are limited to total carbohydrate exclusive of hemicellulose and cellulose, and its major cleavage product—reducing sugar. Nitrogen values are similarly limited to protein and the major cleavage product—amino acid. Values for soluble nitrogen have also been used, particularly if corrected for nitrate.

Four points have been kept in mind by the writer in the evaluation of the numerical data. (*a*) The selected data and the conclusions they entail concerning the individual researches are those of the investigators whose papers are cited. (*b*) The data in these publications have been recomputed for use in these tables and should be compared on a relative and individual basis. That is they should be compared in pairs: free amino nitrogen versus protein, and reducing sugar versus carbohydrate. While many of the tabulated values are also true on an absolute basis, this is considered relatively immaterial from the viewpoint of consecutive chemical reactions. (*c*) Mild or no symptoms are given equal weights in the means and the total number of positive results despite any proof other than the intention of the investigator that a deficiency existed. (*d*) The calculated means and the number of positive results are used as a rough measure of the extent of agreement in investigations. However, the degree of mineral deficiency, the magnitude of differences in pairs of values, and the extent and consistency of the values in each investigation are also used as aids in interpretation.

EFFECTS OF MINERAL DEFICIENCIES ON THE PROTEIN AND AMINO ACID CONTENT OF PLANTS

A summary of the results reported by investigators as concerns the effects of mineral deficiency on protein metabolism is contained in Tables I–III. These results are limited on the whole to leaves and stem. Special procedures of sampling such as the use of fractional parts of the stem are so indicated and enclosed in parentheses; analyses of roots or fruit are also placed in parentheses.

Table I deals with the effects of nitrogen, phosphorus, and potassium deficiency. The values for amino nitrogen in the leaves of nitrogen-deficient plants seem to indicate a slight tendency to increase as compared with the controls. If these ratios are averaged, however, it is seen that the amino acid content of deficient plants is identical with that of normal plants, while protein has decreased by one-third. On a relative basis, however, amino-nitrogen has increased as compared with protein. Analogous values for the stems differ only slightly. Richards and Templeman (*36*) interpret their data on nitrogen deficiency to mean that no marked disturbance of protein metabolism takes place. The quantitative data tabulated for nitrogen deficiency verify the earlier conclusions of Kraybill and Smith (*23*) and Kraybill (*22*). An interesting study of nitrogen deficiency in corn grown with ammonium-nitrogen (*57*) should be consulted. Wood and Petrie (*61*) conclude from their data that the concentration of sugar applied from an external source did not disturb the relation of amino acids to protein in leaves of *Phalaris tuberosa* L. Sucrose, glucose, and fructose were used.

Results on phosphorus deficiency are less ambiguous. A definite increase in amino acid nitrogen and decrease in protein is readily evident both in the leaves and stems. This interpretation is supported by the values of the means and again agrees with the interpretation of Richards and Templeman (*36*). The reasons for the apparent variations in the results of different investigators are not entirely clear, except that those data obtained with material from plants showing severe symptoms of phosphorus deficiency show the greater differences. The phosphorus deficiency data are also in accord with the prior work of Kraybill and Smith (*23*) and Kraybill (*22*) on tomato plants. Those of Turtschin (*53*) and of Smirnov *et al.* (*44*) also show good agreement.

Potassium deficiency studies seem to have been favored by investigators. These have reported, with few exceptions, that insufficiency of potassium causes a disturbance in protein metabolism indicated by a relative increase in amino acid nitrogen and a decrease in protein. Both leaves and stems are affected. Richards and Templeman (*36*) interpret these results as due to breakdown of protein and not an inhibition of

TABLE I

Effects of Mineral Deficiency on Free Alpha-Amino Nitrogen and Protein Nitrogen of Plants: Nitrogen, Phosphorus, Potassium

Plant	Symptoms	Deficiency/Control Ratios				Reference
		Leaves		Stems		
		Amino acid-N*	Protein N	Amino acid-N*	Protein N	
Nitrogen deficiency						
Tung	mild	0.67	0.77			(8)
Barley	medium	1.38	less			(*13*)
Tomato		0.21	0.25	0.18	0.25	(*19*)
Barley	severe	1.41	0.95			(*36*)
Tobacco	severe	1.32				(*50*)
Mean		1.00 (*5*)	0.66 (*3*)	0.18 (*1*)	0.25 (*1*)	
Phosphorus deficiency						
Tomato (NO_3-N)	mild	0.90	1.05	0.95	1.36	(2)
Tomato (Urea-N)	mild	0.74	0.86	1.13	0.80	(2)
Barley	medium	1.10	less			(*13*)
Tomato		0.99	0.78	1.80	0.40	(*19*)
Wheat (tops)	severe	(1.09)^{s}	(0.94)			(*21*)
Wheat (roots)	severe	(1.48)^{s}	(0.78)			(*21*)
Wheat (seed)	severe	(1.28)^{s}	(0.97)			(*21*)
Tomato	medium	0.53^{s}	0.83	0.83^{s}	1.14	(*25*)
Barley	severe	2.09	0.66			(*36*)
Tobacco	mild	1.48				(*50*)
Mean		1.12 (*7*)	0.84 (*5*)	1.18 (*4*)	0.93 (*4*)	
Potassium deficiency						
Soybean	medium	1.38	0.90	1.14	0.72	(*4*)
Guayule (immature)	medium	2.40	1.24			(*7*)
Guayule (mature)	medium	2.98	1.23			(*7*)
Tung	mild	0.83	1.01			(*8*)
Barley	medium	0.64	more			(*13*)
Sugar cane	medium	3.30	0.92	1.12	0.92	(*16*)
Cowpea	mild	1.09	0.94	0.95	1.18	(*18*)
Sugar beet	mild	1.43	1.39			(*18*)
Sugar beet (roots)	mild	(1.12)	(1.10)			(*18*)
Tomato		2.75^{s}	1.04	2.32^{s}	0.85	(*19*)
Tomato	medium	1.38	1.02	1.32	1.07	(*32*)
Tomato	mild	1.29^{s}	1.11	1.23^{s}	1.00	(*35*)
Barley	medium	1.30	0.92			(*36*)
Oats	medium	2.91^{s}	1.46^{s}			(*37*)
Pineapple (NO_3-N)		1.23	1.09	1.22	0.80	(*42*)
Pineapple (NH_4-N)		2.46	1.31	1.82	0.73	(*42*)
Tobacco	severe	6.87				(*50*)
Tomato	medium	1.92^{so}	1.02	1.73^{so}	1.21	(*59*)
Mean		2.13 (*17*)	1.04 (*16*)	1.43 (*9*)	0.94 (*9*)	

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*Values representing soluble nitrogen are indicated by "s," and soluble organic nitrogen by "so."

synthesis. Other data (*50*) clearly indicated that the magnitude in the difference obtained depends on the severity in deficiency symptoms and the degree of exclusion of apparently normal tissues. That is to say, that both the symptoms and the degree of chemical change are localized. Nevertheless, Phillips, Smith, and Dearborn (*34*) were unable to find any indication of derangement of nitrogen metabolism with potassium-deficient tomato plants.

TABLE II

Effects of Mineral Deficiency on Free Alpha-Amino Nitrogen and Protein Nitrogen of Plants: Magnesium, Calcium, Sulfur

Plant	Symptoms	Deficiency/Control Ratios				Reference
		Leaves		Stems		
		Amino acid-N	Protein N	Amino acid-N	Protein N	
Magnesium deficiency						
Soybean		1.36	0.85	0.71	0.91	(*4*)
Tobacco	severe	3.83				(*50*)
Mean		2.60 (2)	0.85 (*1*)	0.71 (*1*)	0.91 (*1*)	
Calcium deficiency						
Soybean	mild	0.63	0.65	0.68	0.67	(*4*)
Tomato (–Cu also)				0.93	1.03	(*31*)
Tobacco	severe	2.20				(*50*)
Mean		1.42 (2)	0.65 (*1*)	0.81 (2)	0.85 (*1*	
Sulfur deficiency						
Soybean	medium	1.15	0.87	2.61	1.51	(*9*)
Soybean (roots)	medium	(2.02)	(0.71)			(*9*)
Sunflower (upper)	medium			4.31	0.85	(*10*)
Sunflower (middle)	medium			5.43	0.71	(*10*)
Sunflower (lower)	medium			2.74	0.77	(*10*)
Tomato	slight			3.88	0.90	(*33*)
Mean		1.15 (*1*)	0.87 (*1*)	3.79 (5)	0.99 (5)	

Deficiences in magnesium, calcium, and sulfur (Table II) seem to have an identical influence on the relative quantities of amino acid nitrogen in plants. Most investigations agree in reporting an increase in free amino acids in the leaves of plants deficient in either magnesium, calcium, or sulfur. However, no significant change is reported by

Burrell (*4*) as taking place in the stems of soybean with either calcium or magnesium deficiency. Nightingale, Addoms, Robbins, and Schermerhorn (*31*) also found little alteration in amino acid and protein content of tomato stems to occur with calcium deficiency. The data for sulfur deficiency in stems showed more differences in all cases in regard to both chemical reactions.

The reason for the unchanged values in stems with magnesium and calcium deficiency might be one of several. It may be a characteristic reaction to a deprivation of these elements. A mild deficiency could also possibly cause a similar condition. Further, it should be recognized that, if localization of symptoms is paralleled by alterations in amino acid and protein content, the stems of plants should be relatively less affected than the leaves.

Skok (*43*) obtained calcium deficiency symptoms with the bean plant when supplied with urea as a source of nitrogen. Additional studies would seem desirable, but it seems clear that nitrate reduction is not a primary function of calcium, nor the inactivation of nitrate a causative factor in calcium deficiency.

The data on the effects of deficiencies in micronutrients are very few (Table III). Bennett (*1*) reports that iron deficiency leads to an increase in amino acid nitrogen and a decrease in protein nitrogen. Gilbert, Sell, and Drosdoff (*12*) reported a definite increase in both constituents in the leaves of tung during early stages of growth with copper deficiency. Later stages showed a marked decrease in free amino acids, however. These results may afford an explanation of those reported by Lucas (*24*), who concluded that copper does not participate in protein metabolism. The increased protein may well be due to an inhibition in protein breakdown, a view in accord with that of Gregory and Sen (*14*) that synthesis and breakdown of protein followed different paths. Copper enzymes may play a relatively small part in the former reaction as compared with the latter.

The data of Mulder (28) on molybdenum deficiency are different for leaves and stems. This author emphasizes the decrease in protein accompanying a molybdenum deficiency and the large increases in unutilizable nitrate. The leaves show a relatively lesser decrease in amino acids than in protein.

Insufficient data on the effects of boron deficiency also do not permit

TABLE III

Effects of Mineral Deficiency on Free Alpha-Amino Nitrogen and Protein Nitrogen of Plants: Iron, Copper, Molybdenum, Boron

Plant	Symptoms	Deficiency/Control Ratios				Reference
		Leaves		Stems		
		Amino acid-N*	Protein N	Amino acid-N*	Protein N	
Iron deficiency		+	−			(*1*)
Copper deficiency						
Tung (early)	mild	1.89	1.46			(*12*)
Tung (late)	mild	0.43	1.32			(*12*)
Alfalfa			1.09			(*24*)
Barley			1.43			(*24*)
Oats			1.13			(*24*)
Wheat			1.04			(*24*)
Wheat (grain)			(1.11)			(*24*)
Sugar beet			1.23			(*24*)
Tomato (fruit)			(1.14)			(*24*)
Carrot (root)			(1.48)			(*24*)
Mean		1.16 (*2*)	1.24 (*7*)			
Molybdenum deficiency						
Tomato	mild	0.96	0.83	0.61	0.78	(*28*)
Tomato (roots)		(0.84)	(1.07)			(*28*)
Mean		0.96 (*1*)	0.83 (*1*)	0.61 (*1*)	0.78 (*1*)	
Boron deficiency						
Nasturtium	medium	0.70^{so}	0.72	1.08^{so}	0.78	(*3*)
Nasturtium (roots)		$(0.65)^{so}$	(0.76)			(*3*)
Alfalfa (tops)	medium	$(1.69)^{so}$				(*38*)
Spinach	mild	$(1.06)^{s}$	(0.89)			(*39*)
Tobacco	mild	1.27				(*50*)
Mean		0.99 (*2*)	0.72 (*1*)	1.08 (*1*)	0.78 (*1*)	

*Values representing soluble nitrogen are indicated by "s," and soluble organic nitrogen by "so."

a clear cut interpretation. Scripture and McHargue reported increased free amino acids in alfalfa tops (*38*) and spinach (*39*) and a drop in protein in the latter. Briggs (*3*) found amino acid and protein nitrogen decreased to about the same extent in the leaves of the nasturtium plant, whereas the former increased and the latter decreased in the stems. A

TABLE IV

Effects of Mineral Deficiency on Reducing Sugars and Total Carbohydrates (sugars plus starch) of Plants: Nitrogen, Phosphorus, Potassium

Plant	Symptoms	Deficiency/Control Ratios				Reference
		Leaves		Stems		
		Reducing sugar	Carbohydrate*	Reducing sugar	Carbohydrate*	
Nitrogen deficiency						
Tung		0.89	0.93			(*8*)
Barley	mild	1.08	1.78^{s}			(*13*)
Tomato	severe	0.36	1.55	0.59	0.93	(*19*)
Mean		0.78 (*3*)	1.24 (*2*)	0.59 (*1*)	0.93 (*1*)	
Phosphorus deficiency						
Barley	mild	1.33	1.10^{s}			(*13*)
Lemon		0.73	0.51^{s}	0.14	0.19	(*15*)
Tomato	medium	0.24	1.09	0.87	0.95	(*19*)
Wheat (dark-7 days)		1.29	1.29			(*21*)
Wheat (root-seed)		(0.79)	(0.79)	(1.35)	(1.01)	(*21*)
Tomato (average)	medium	1.63	0.86	1.79	1.34	(25)
Mean		1.04 (5)	1.08 (*3*)	0.93 (*3*)	0.69 (*3*)	
Potassium deficiency						
Soybean		0.83	1.24	0.58	0.64	(*4*)
Guayule (immature)	medium	2.39	1.47	3.46	1.09	(7)
Guayule (mature)	medium	3.41	1.06	0.96	0.63	(7)
Tung	moderate	0.75	0.90			(*8*)
Barley	mild	0.80	0.63^{s}			(*13*)
Sugar cane (9 week)	medium	1.10	0.96^{s}	0.85	1.08^{s}	(*16*)
Sugar cane (7½ month)	medium	1.17	1.02^{s}	0.43	0.65^{s}	(*16*)
Pea	mild	0.67	1.03	0.80	0.90	(*17*)
Cowpea		1.28	1.11	1.62	1.27	(*18*)
Sugar beet (roots)		0.80	0.87	(0.70)	(0.65)	(*18*)
Tomato	mild	0.66	0.62	0.86	0.83	(*19*)
Tomato	medium	0.58	0.47	1.16	0.75	(32)
Tomato		0.91	1.10	1.24	1.13	(35)
Pineapple (NO_3-N)		1.96	1.41	1.17	0.66	(*41*)
Pineapple (root)		(3.11)	(1.84)			(*41*)
Pineapple (NH_4-N)		2.35	0.92	1.47	0.61	(*41*)
Pineapple (root)		(1.19)	(0)			(*41*)
Tomato	severe	1.76	1.33	1.31	0.81	(59)
Tomato (nitrate)	medium		1.09		1.09	(60)
Tomato (ammonia)	medium		0.84		0.82	(*60*)
Mean		1.34 (*16*)	1.03 (*15*)	1.22 (*13*)	0.86 (*13*)	

*Values for total sugar are indicated by "s."

parallelism between severity in symptoms and degree of chemical change has been noted for boron by Steinberg, Bowling, and McMurtrey (50). A microchemical study by Wadleigh and Shive (58) led these investigators to conclude that boron deficiency caused an alteration in the normal course of protein synthesis in cotton seedlings.

EFFECTS OF MINERAL DEFICIENCIES ON THE REDUCING SUGAR AND CARBOHYDRATE CONTENT OF PLANTS

Nitrogen, phosphorus, and potassium deficiencies in Table IV also reflect the effects of divergencies in sampling and climatic conditions. However, certain variations in composition have been noted by investigators in their experiments. These changes consist, with nitrogen deficiency, of a relative decrease in reducing sugar and an increase in total carbohydrate. The increase in carbohydrate is attributed to diminished formation of protein. Phosphorus deficiency leads to equal increases in both fractions for the same reason, while lack of potassium causes a greater increase in reducing sugar than in total carbohydrate.

The mean values for nitrogen deficiency in Table IV indicate a relative decrease in reducing sugar as compared with total carbohydrate both in leaves and stems. Reducing sugar apparently increased relatively to carbohydrate only in the stems of plants subject to phosphorus deficiency, but not in the leaves. MacGillivray's data (Table VII) would indicate this to be only an apparent exception. With potassium deficiency, reducing sugars again seemed to show a relative increase as compared with carbohydrate in both stems and leaves on the basis of the mean values.

Analytical data on the contents of reducing sugar and carbohydrate are very limited in the cases of magnesium, calcium, and sulfur deficiencies. Table V presents the available data. Almost invariably there is a decrease in both fractions in both leaves and stems with these three elements. Only in the case of stems short of calcium is there a slight increase in both reducing sugar and total carbohydrate.

Micronutrient deficiency effects on reducing sugar and carbohydrate have been tabulated in Table VI. Iron-deficient pineapple plants are lower in total carbohydrates than are the controls and this is accompanied by a slight relative increase in reducing sugar. Both leaves and

TABLE V

Effects of Mineral Deficiency on Reducing Sugars and Total Carbohydrates (sugars plus starch) of Plants: Magnesium, Calcium, Sulfur

Plant	Symptoms	Deficiency/Control Ratios				Reference
		Leaves		Stems		
		Reducing sugar	Carbohydrate	Reducing sugar	Carbohydrate	
Magnesium deficiency						
Soybean	mild	0.88	0.85	0.73	0.74	(4)
Mean		0.88	0.85	0.73	0.74	
Calcium deficiency						
Soybean		1.11	1.08	1.10	0.91	(4)
Pea		0.47	0.70	0.40	0.72	(17)
Tomato				1.94	2.07	(31)
Mean		0.79 (2)	0.89 (2)	1.15 (3)	1.23 (3)	
Sulfur deficiency						
Soybean	medium	0.84	0.84	0.18	1.44	(9)
Soybean (roots)	medium	(0.67)	(1.20)			(9)
Sunflower				0.23	0.27	(10)
Mean		0.84	0.84	0.21 (2)	0.86 (2)	

stems responded similarly. Copper deficiency had a similar effect on leaves of the tung tree. Withholding boron, however, led to increases in total carbohydrates in leaves and stems, with relatively increased reducing sugar in the former and a decreased content in the latter.

SOME DATA OF INDIVIDUAL INVESTIGATIONS

A clearer idea of the meaning of the preceding summaries can be obtained through an examination of some of the specific data. The following have been selected on the basis of consistency in results, size of plant samples, and comparison of effects in different organs in the plant. Data meeting these criteria are confined almost entirely to nitrogen, phosphorus, and potash. Attention is again called to the fact that these data have been recomputed in terms of "quantity in deficient plant/quantity in the control."

TABLE VI

Effects of Mineral Deficiency on Reducing Sugars and Total Carbohydrates (sugars plus starch) of Plants: Iron, Copper, Boron

Plant	Symptoms	Deficiency/Control Ratios				Reference
		Leaves		Stems		
		Reducing sugar	Carbo-dyhrate*	Reducing sugar	Carbohydrate	
Iron deficiency						
Pineapple (NO_3-N)		1.04	0.89	0.90	0.84	(40)
Pineapple (NH_4-N)		0.74	0.80	1.02	0.77	(40)
Mean		0.89	0.85	0.96	0.81	
Copper deficiency						
Tung (early)	mild	0.77	0.79			(*12*)
Tung (late)	mild	0.97	0.83			(*12*)
Mean		0.87	0.81			
Boron deficiency						
Tomato	medium	2.14	1.73	0.61	1.06	(*20*)
Tomato (roots)	medium	(2.09)	(1.85)			(*20*)
Alfalfa (tops)	medium	(1.85)	(1.66)^{8}			(*38*)
Mean		2.14	1.73	0.61	1.06	

*Values for total sugar indicated as "s."

The effects of phosphorus deficiency on carbohydrate metabolism of the tomato plant obtained by MacGillivray (*25*) are illustrated in Table VII. Phosphorus deficiency caused a relative increase in reducing sugars in both leaves and stems. Whereas total carbohydrates—sugars plus starch—increased to a somewhat lesser extent also in the stems, a definite decrease took place in the leaves. There was on the whole a tendency for all carbohydrate to concentrate in the lower portions of the plant. Some degree of correlation exists therefore between the loci of symptoms and of greatest variations in reducing sugars and total carbohydrates.

The data of Nightingale, Schermerhorn, and Robbins (*32*) on the effects of potassium deficiency on the tomato plant are shown in Table VIII and include values for amino and protein nitrogen. Reducing

TABLE VII

Effect of Phosphorus Deficiency on the Carbohydrate Content of the Tomato Plant*

Plant Part	Deficiency/Control Ratios: Phosphorus	
	Reducing sugar	Carbohydrates†
Leaves		
Top	1.16	0.71
Next to top	1.03	0.45
Middle	1.52	0.74
Next to bottom	2.82	1.54
Mean	1.63	0.86
Stems		
Next to top	1.65	0.98
Middle	1.86	1.28
Next to bottom	1.99	1.55
Bottom	1.67	1.55
Mean	1.79	1.34

*Computed from data of MacGillivray (25)
†Sugars plus starch

TABLE VIII

Effect of Potassium Deficiency on Protein and Carbohydrate Content of the Tomato Plant*

Plant Part	Deficiency/Control Ratios: Potassium			
	Amino-N	Protein-N	Reducing sugar	Carbohydrate
Upper blades	1.34	0.97	0.39	0.39
Lower Blades	1.41	1.07	0.60	0.48
Upper petioles	2.57	0.92	0.67	0.67
Lower petioles	1.24	0.71	0.64	0.34
Upper stems	1.35	0.98	1.07	0.83
Lower stems	1.29	1.16	1.25	0.66
Roots	1.60	1.00	0.97	0.84

*Computed from data of Nightingale, Schermerhorn, and Robbins (32) sugars in the deficient plants decreased in the leaves and increased in the stems. Total carbohydrate gave consistent decreases in all parts of the plant. The net result was a relative increase in reducing sugars as compared with total carbohydrate because of potassium deficiency. Carbohydrates tended to increase in the lower parts of the plant, where symptoms of potassium deficiency are most extreme.

Protein content also seems to become higher in the lower leaves and stems of plants lacking in potassium. Amino nitrogen shows a definite increase as compared with normal plants, due either to proteolysis or more probably to a block in protein formation. The quantities of all constituents parallel each other in kind as between upper and lower leaves. The effects of deficiency in causing a relative increase in breakdown products—amino acids and reducing sugars—are plainly visible in the roots.

The sampling procedure followed by Richards and Templeman (*36*) and Gregory and Baptiste (*13*) depended on the analysis of successive leaves of barley plants as each reached maturity. The effects of deficiencies would be expected to increase in severity with successive leaves unless the element lacking was rapidly and completely mobile.

Examination of the data of Richards and Templeman (*36*) in Table IX discloses that the relative quantities of amino and protein nitrogen remained unaltered by nitrogen deficiency; whereas the former showed marked increases with phosphorus and potassium deficiencies. These displacements in relative quantities were caused in part by a relative fall in protein with insufficient phosphorus. Protein apparently did not decrease with potassium deficiency. These authors concluded that phosphorus was necessary for the formation of protein, and that potassium did not participate in this process. The increased amino acid content of leaves lacking potassium in barley was attributed to proteolysis.

The results for carbohydrates with barley plants subject to nitrogen, phosphorus, and potassium deficiency have been reported by Gregory and Baptiste (*13*). Some of their data have been tabulated in Table X. Total carbohydrates were not determined, unfortunately. Total sugars seemed to show an increase with deficiencies in nitrogen and phosphorus, but to diminish sharply with lack of potassium.

TABLE IX

Effect of Nitrogen, Phosphorus, and Potassium Deficiency on the Protein Content of Consecutively Matured Leaves of Barley*

Leaf Number from Base to Tip	Deficiency/Control Ratios					
	Low nitrogen		Low phosphorus		Low potassium	
	Amino-N	Protein-N	Amino-N	Protein-N	Amino-N	Protein-N
1	1.00	0.99	1.11	0.97	1.00	0.97
2	0.99	1.00	0.98	0.78	1.01	0.84
3	0.96	1.40	1.04	1.42	1.11	1.51
4	0.90	0.95	0.90	0.91	0.97	0.95
5	0.68	0.50	1.55	0.72	1.22	0.87
6	0.53	0.41	1.32	0.64	1.30	0.96
7	0.40	0.29	2.16	0.69	1.55	0.86
8	0.47	0.38	1.58	0.93	1.58	1.10
9	0.52	0.53	1.54	0.85	2.04	0.95
10	—	0.57	3.24	0.94	3.01	1.10
Mean	0.72	0.70	1.54	0.89	1.48	1.01

*Computed from data of Richards and Templeman (*36*)

TABLE X

Effect of Nitrogen, Phosphorus, and Potassium Deficiency on the Carbohydrate Content of Consecutively Matured Leaves of Barley*

Leaf Number from Base to Tip	Deficiency/Control Ratios					
	Low nitrogen		Low phosphorus		Low potassium	
	Reducing sugar	Sugar	Reducing sugar	Sugar	Reducing sugar	Sugar
1	1.20	0.92	1.08	0.90	1.08	0.95
2	1.18	2.15	0.97	1.93	0.82	0.94
3	0.99	1.06	0.94	1.01	1.14	0.87
4	1.02	1.23	0.86	0.72	0.85	0.43
5	1.10	2.01	1.45	1.41	0.98	0.89
6	0.67	1.76	1.27	0.57	0.49	0.21
7	—	—	—	—	0.54	0.38
8	0.52	1.16	0.59	0.66	0.35	0.30
9	1.14	1.98	1.16	0.93	0.79	0.59
10	1.59	1.04	—	—	—	—
Mean	1.05	1.48	1.04	1.02	0.78	0.62

*Computed from data of Gregory and Baptiste (*13*)

The data obtained by Wall (59) on potassium shortage of the tomato plant (Table XI) agree with those of Richards and Templeman in Table IX in regard to the disproportionate increase in amino acids and maintenance in protein content. They differ from those of Gregory and Baptiste (Table X) in showing both an increase in total carbohydrate and the relatively increased content of reducing sugars. Wall pointed out, therefore, that the evidence favored potassium participation in protein formation, since proteolysis does not ordinarily occur in the presence of adequate sugar.

TABLE XI

Effect of Potassium Deficiency on the Protein and Carbohydrate Content of the Tomato Plant*

Plant Part	Deficiency/Control Ratio: Potassium			
	Soluble Organic-N	Protein-N	Reducing sugar	Carbo-hydrate†
Leaves				
Upper	1.86	1.16	1.03	0.87
Lower	1.98	0.87	3.93	2.85
Mean	1.92	1.02	2.48	1.86
Stems				
Upper	1.90	1.37	0.69	0.72
Lower	1.56	1.04	1.16	0.55
Mean	1.73	1.21	0.93	0.64

*Computed from data of Wall (59)
†Sugars plus starch

In these experiments sugars and carbohydrates were higher in the lower parts of the plant, while amino acids and protein tended to be greater in the younger tissues. The nitrogen differences were slight, however.

The experimental results of Sideris and Young have interested the writer for they afford a comparison between the effects of ammonium and nitrate nitrogen on potassium deficiency. The analytical results for potassium deficiency in the pineapple are shown in Table XII (*41*, *42*). Here again will be seen a relative increase in amino acids and reducing sugars in all parts of the plants supplied with nitrate nitrogen or with ammonium nitrogen. Protein has increased in the leaves but diminished in the stems. It may be noted here that the assumption that potassium deficiency owes its action to a "locking" of nitrate (*11*) is not borne out by the appearance of potassium deficiency symptoms in the absence of nitrate nitrogen.

TABLE XII

Effect of Potassium Deficiency on the Protein and Carbohydrate Content of the Pineapple*

Plant Part	Deficiency/Control Ratios: Potassium							
	Nitrate-N				Ammonium-N			
	Amino-N	Protein-N	Reducing sugar	Carbohydrate†	Amino-N	Protein-N	Reducing sugar	Carbohydrate†
Leaves								
Young	1.13	1.09	1.63	1.17	2.13	1.12	1.03	0.89
Active	1.43	1.19	1.93	1.74	2.20	1.18	1.05	1.12
Mature	1.41	1.11	2.59	1.56	2.93	1.50	1.39	1.10
Old	1.23	0.94	1.92	1.51	2.52	1.32	1.30	1.12
Mean	1.30	1.09	2.02	1.50	2.46	1.31	1.19	1.06
Stems								
Apex	1.38	0.79	1.88	2.20	2.05	0.57	1.10	2.13
Middle	1.22	0.74	2.31	0.56	1.98	0.74	1.81	0.61
Base	1.09	0.87	2.06	0.37	1.39	0.86	1.74	0.60
Mean	1.24	0.80	2.08	1.04	1.82	0.73	1.46	1.11
Roots	1.73	0.80	3.11	1.78	1.02	0.78	1.19	—

*Computed from data of Sideris and Young (*41*, *42*)
†Sugars plus starch

Definite gradients in ratios may be noted in the pineapple plant that has a potassium deficiency. The values for young and old leaves tend to show a lesser increase in nitrogen and carbohydrate constituents. Amino acids and carbohydrates decrease from the apex to the base of the stem.

The data in Table XIII are included to illustrate the relative increase in reducing sugar as compared with total carbohydrate in the iron deficiency of the pineapple plant. A similar response was obtained with ammonium and nitrate nitrogen.

TABLE XIII

Effect of Iron Deficiency on the Carbohydrate Content of the Pineapple*

Plant Part	Deficiency/Control Ratios: Iron			
	Nitrate-N		Ammonium-N	
	Reducing sugar	Carbohydrate†	Reducing sugar	Carbohydrate†
Leaves				
Young	1.06	0.93	0.78	0.87
Active	0.92	0.80	0.63	0.67
Mature	0.85	0.79	0.84	0.82
Old	1.29	1.03	1.06	0.84
Mean	1.03	0.89	0.83	0.80
Stems				
Apex	0.86	0.66	1.10	0.82
Middle	0.92	0.89	1.04	0.69
Base	0.93	0.93	0.94	0.79
Mean	0.90	0.83	1.03	0.77
Roots	0.67	1.02	0.76	0.75

*Computed from the data of Sideris and Young (*40*)
†Sugars plus starch

CHEMICAL MECHANISM OF VISUAL SYMPTOM PRODUCTION

The writer has reported (*48*) that characteristic responses in gross morphology of tobacco seedlings take place in aseptic culture on a complete mineral agar with traces of each of the free amino acids. Isoleucine in quantities of 20–100 p.p.m. caused production of frenching-like symptoms including network chlorosis, inhibition of stem and branch elongation, abnormal increase in leaf number, and inhibition of leaf lamina growth. Figures 2 and 3 illustrate frenching of tobacco in the field and the isoleucine effect in aseptic culture, respectively.

Hydroxyproline at 5 p.p.m. quickly killed. Various types of chloroses, necroses, and abnormalities in leaf form were characteristic of other

FIGURE 2. Field of tobacco in Lancaster County, Pennsylvania, showing extreme frenching.—Courtesy of Dr. O. E. Street.

amino acids. The conclusion was drawn that production of symptoms in the plant was probably due to abnormal protein metabolism and the excessive accumulation of metabolites. A later paper (*49*) confirmed and extended these results to possible aliphatic acid metabolites. Only the natural isoleucine was found to be effective, and not the unnatural optical isomer (Figure 4).

A still later paper (*50*) dealing with field plants of tobacco showing symptoms of frenching and mineral deficiencies affords additional evidence for this interpretation. Symptoms of frenching and of calcium, magnesium, potassium, phosphorus, and boron deficiency were accompanied in each case by marked increases in free amino acid content of the leaf lamina. These increases ranged up to about 600 per cent:

FIGURE 3. Maryland Medium Broadleaf tobacco in asceptic culture illustrating the effect of 200 p.p.m. of synthetic isoleucine.

nitrogen, 32; phosphorus, 48; potassium, 587; calcium, 120; magnesium, 283; boron, 27; and frenching, 94. Chloroses were attributed primarily to the accumulation of toxic metabolic products and were not, except possibly magnesium, evidence of a function in chlorophyll formation.

An important corollary of these results has a direct bearing on the

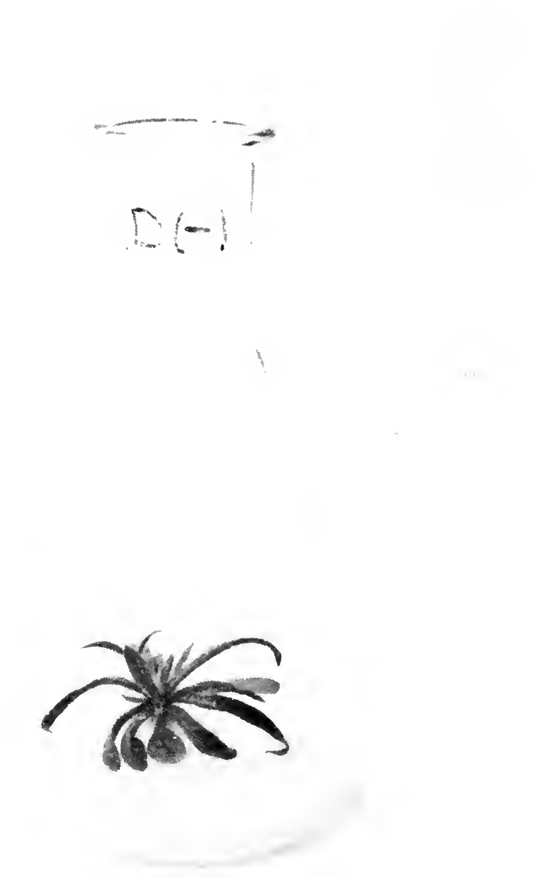

FIGURE 4. Maryland Medium Broadleaf tobacco in aseptic culture illustrating the effect on growth of 100 p.p.m. of natural isoleucine. The "D(—)" designation is an error. It should be "L(+)."

preceding data concerned with the metabolic changes accompanying mineral deficiencies. Alterations in metabolism of the leaf lamina were found to be proportional to the degree of abnormality. Localization, it was surmised, operates even in individual leaves, the healthy portions showing relatively little change compared to abnormal portions.

DISCUSSION

The summary of data in the literature concerning the effect of mineral deficiency on amino acids and protein shows how greatly the results of various investigators have differed. A certain degree of uniformity has been introduced, perhaps unwarranted, by comparing the mean values obtained in all experiments. Another procedure, namely, comparison of the number of positive results with negative results, might also have been used. A brief computation will disclose that six out of nine of the investigators with phosphorus, and fifteen of seventeen with potassium obtained an increase in amino acids and a relative decrease in protein of the leaves with deficiency. Sulfur deficiency resulted in similar reactions without exception. Since the greater number of experiments deal with potassium and phosphorus in the order given and only limited information is available for the other elements, these may be taken to indicate that any mineral deficiency results in a disturbance of protein metabolism. Only in a few instances did the analytical data indicate an unchanged proportion of amino acids to protein in either leaves or stems as a result of deficiency.

This interpretation is also supported by the data of those experimental results of greater uniformity due in part to the use of large samples—for example, those of Sideris and Young (*40, 41*), Nightingale *et al.* (*31, 32*) and Wall (*59, 60*). Steinberg, Bowling, and McMurtrey (*50*) were able to demonstrate that marked increases in free amino acids took place in leaves of field-grown tobacco. Large samples of 50 to 100 leaves were used and positive results were obtained in each case (calcium, magnesium, potassium, phosphorus, nitrogen, and boron) in accordance with the severity of symptoms displayed.

The mean values for nitrogen, phosphorus, and potassium deficiency in successive leaves as they matured were determined by Richards and Templeman (*36*) and are shown in Table X. Nitrogen deficiency did not alter the relative proportion of amino acid and protein. Phosphorus deficiency caused a rise in amino acids and a fall in protein. Potassium deficiency also led to a rise in amino acids, while protein remained unchanged. It might be noted in this connection that the authors' conclusion that increased amino acids with potassium deficiency are the result of increased hydrolysis and not hindrance in synthesis of protein is not supported by their data. Wall (*59*) discussed this point also and disagreed with their interpretation particularly because of the presence of ample sugar in the leaves to prevent hydrolysis.

Another interesting comparison of these data is one based on the hypothesis that mineral deficiency leads to a disproportionate content of amino acids and reducing sugar as compared with normal plants. Computation of the average deficiency–control ratios for all chemical elements discloses that in 9 out of 10 instances amino nitrogen was relatively greater than protein nitrogen in leaves, and only 4 out of 8 times in the stems. The analogous figures for reducing sugar and carbohydrate were 5 out of 8 in leaves, and 3 out of 8 in the stems. Since symptoms of abnormal nutrition are primarily localized in the leaves, we may assume that mineral deficiencies lead to an accumulation of amino acids as compared to protein, and of reducing sugar as compared to carbohydrate. Extent and duration of deficiencies will probably be found to cause progressive and opposite reactions in proportions of carbohydrates.

An accumulation of nitrate in deficient plants appears to be a general phenomenon of mineral deficiency excepting nitrogen and boron. Since deficiency symptoms appear in plants supplied with ammonium nitrogen, it must be assumed that inability to utilize nitrate is a result and not a cause of symptoms. With the fungus, *Aspergillus niger*, only in the case of molybdenum has a mineral deficiency been found dependent on the source of nitrogen (*45*). Further studies with green plants will presumably lead to similar results, inasmuch as Mulder (*28*) was able to demonstrate a diminution effect of ammonium nitrogen on molybdenum deficiency in tomatoes, barley, and oats.

In the tung and other trees, however, nitrate reduction apparently occurs in the roots instead of the leaves, and no evidence has as yet been found that mineral deficiency can cause an accumulation of nitrate. Nitrates, it is known, can be caused to accumulate in leaves of herbaceous plants by manipulation of climatic conditions irrespective of mineral deficiencies. It is evident, therefore, that caution should be exercised in attributing the accumulation of an intake material or metabolite primarily to mineral nutrition. Such an accumulation may be due instead to a diminution in demand due to a decreased rate of growth.

The experimental results here summarized have been obtained almost wholly with herbaceous plants; the pineapple, guayule, and tung tree are the only exceptions. Analytical results for the tung tree disagreed in many respects with those for herbaceous plants. A decision on whether this disagreement is caused by an intrinsic difference in metabolism between these two types of vegetation must await the accumulation of data on a large variety of trees.

SUMMARY

Mineral deficiencies in plants appear to lead to a relative accumulation of amino acids and reducing sugars. Protein may or may not decrease depending on the extent and duration of deficiencies. Accumulation of nitrates appears to be a result of diminished growth with mineral deficiency, except in the case of molybdenum. The alterations in proportions of nitrogen fractions lead to the assumption that all minerals participate directly or indirectly in nitrogen metabolism. Carbohydrates may temporarily accumulate during the early stages of deficiency. Evidence exists for a localization effect in that plant parts show a degree of parallelism between severity of visual and chemical symptoms. It appears useless to attempt to draw any connection between individual deficiencies and physiological responses in the plant, since protein metabolism is a basic reaction for all.

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MODIFYING INFLUENCES OF VARIOUS ENVIRONMENTAL FACTORS UPON MINERAL NUTRITION

CHAPTER

16 Light as a Modifying Influence on the Mineral Nutrition of Plants

ROBERT B. WITHROW

LIGHT is not known to play any direct role indispensable for the absorption, movement, or metabolism of the mineral nutrients. The ions of the mineral macroelements in the inorganic state have no absorption bands making it possible for direct light interaction. A few of the microelements in the ionized state, such as iron, manganese, and copper, have absorption bands in the visible spectrum, but thus far no strong evidence has appeared that light absorption by such ions is of any physiological significance. The effect of light on mineral nutrition, therefore, must be chiefly an indirect one resulting from the temperature rise concomitant with the absorption of light and from the basic photochemical reactions occurring in plants, such as photosynthesis, chlorophyll synthesis, photomorphogenesis, and photoperiodism.

In many cases, the various mineral elements are essential for a photochemical process, either as a constituent of an enzyme system or as a component of a pigment or other substance involved in the photochemical process. In such cases, it is apparent that the rate of the photochemical process frequently will influence the mobilization and utilization of the essential mineral elements. In addition, light can affect the physiology of growth, of which mineral metabolism is a part, through various rather poorly understood photochemical effects such as alteration of permeability, changes in protoplasmic streaming, and various photochemical oxidations.

The most conspicuous indirect role of light in mineral metabolism is through photosynthesis. A carbohydrate supply is necessary as a source of energy for the accumulation of mineral nutrients by roots and for carrying out all other chemical reactions driven energetically by respiratory reactions. The mineral elements enter into synthesis with carbohydrate-derived materials to form the metabolic and structural components of plants. It therefore follows that the mineral nutrient requirement of plants is directly dependent upon the carbohydrate supply and, in turn, upon photosynthesis.

While there are many photochemical reactions in plants necessary for normal growth and development, photosynthesis is the principal energy-converting reaction and consequently requires relatively high light intensities to maintain optimum growth. So far as is now known, all the other photochemical reactions are saturated at relatively low light intensities of the order of 20 foot-candles or less. In the case of photosynthesis in higher plants, on the other hand, saturation does not occur until light intensities of several thousand foot-candles have been attained. In the case of wheat, Hoover *et al.* (*22*) have shown that the relation between the rate of photosynthesis and light intensity is very nearly a linear one up to about 1000 foot-candles at normal atmospheric carbon dioxide concentrations of 0.03 volume per cent. Above 1000–2000 foot-candles, photosynthesis increases with increase in light intensity, but less rapidly than at lower values.

For the other photochemical processes such as chlorophyll synthesis, photoperiodism, and phototropism, the intensity of daylight between sunrise and sunset seldom becomes a factor critically limiting the rate of reaction. For photoperiodism and certain morphogenic effects, the daylight factor that becomes limiting is the duration of the lighted period.

In view of these considerations, the relative length of day and night and incident solar energy become important in relation to mineral nutrition. At 40 degrees latitude in the temperate zone, the natural day length between sunrise and sunset varies from about nine hours to a little over fifteen hours. The range is greater in the more northern latitudes and less in the southern regions. The average daily total solar and sky radiation on a horizontal surface at a 40-degrees latitude station (*19*), however, is 80 per cent less during the winter months than during June and July. Since the reduction in day length is only about 40 per cent, an increase in cloudy weather during the winter accounts for much of the decrease in available radiation. However, data compiled by Kimball (27) have shown that the maximum intensity on a clear day in December is within 10 per cent of the highest intensities attained in June. This generalization applies to data for clear cloudless days from Alaska to Puerto Rico. Thus, greenhouse crops in the winter in the midwestern and northeastern portions of the United States are subjected to short days and relatively low daylight intensities, both of which greatly limit the nutrient requirements of the plants.

THERMAL EFFECTS

The absorption of sunlight by leaves and other portions of the shoot and by the soil surface frequently results in shoot and root temperatures significantly higher than the ambient air temperature. This rise in temperature due to the absorption of radiant energy may become sufficiently high to significantly accelerate thermal reactions and has been shown to alter markedly the transpiration rate; it undoubtedly affects enzymatic reactions and causes changes in form and rate of growth. When means of heat dissipation are restricted, lethal temperatures may be reached.

Horizontal leaf surfaces, at midday during clear weather in the temperate zone, are exposed to sunlight intensities ranging from 1.2 to 1.5 gram calories per square centimeter, minute. And of this total incident energy, 20 to 30 per cent is reflected by thin leaves, a much smaller proportion is transmitted, and the remainder is absorbed. Most of the absorbed energy is degraded to heat and manifests itself as a temperature rise of the exposed portions of the shoot, while only a small portion, less than 5 per cent, is used in photosynthesis.

Curtis (9) has found that the temperature of citrus leaves in intense sunlight may be 10 to 15 degrees above the air temperature and that all leaves exposed to intense sunlight had a higher temperature than that of the surrounding air, regardless of wind velocity or relative humidity, although high wind velocities could reduce the temperature by a matter of 10 degrees. Miller and Saunders (35) have reported a diurnal variation in leaf temperature and transpiration rate of corn, both of which attain maxima coinciding with the maximum of sunlight intensity.

The marked effect of temperature is shown by the fact that a 5-degree rise in temperature of the saturated intercellular spaces of the leaf has the same effect in accelerating evaporation as a 30 to 40 per cent fall in relative humidity (*9*).

Increased transpiration usually results in some increased absorption of mineral nutrients. Freeland (*12*) (Table I), Wright (*59*), and others have shown that the uptake of the macroelements is directly correlated with the transpiration rate, but is not necessarily proportional to the rate of water uptake. Broyer and Hoagland (*4*) have emphasized that the metabolic condition of the plant is a very important factor in determining the influence of transpiration on the uptake of salts by roots. The salt uptake of young barley plants having an initially low salt, high sugar composition was found to be principally dependent on aeration and temperature, with the data showing only a slight increase in salt uptake when the transpiration rate and water absorption were increased by light and low relative humidity (Table II). High salt and low sugar plants, on the other hand, took up potassium and bromium ions at a considerably higher rate as the rate of transpiration and water absorption was increased. These data indicate that water uptake by roots and salt absorption are relatively independent processes, but, under internal root conditions which are unfavorable for salt uptake (as in high salt, low sugar plants), an increased transpiration stream contributes materially to increased salt uptake.

TABLE I

Data on the Amount of Mineral Absorption in Plants with High Transpiration (H.T.) and Low Transpiration (L.T.) (from R. O. Freeland)

		Total Minerals Absorbed, g.	Calcium Absorbed, g.	Phosphorus Absorbed, g.	Potassium Absorbed, g.	Water Absorbed, cc.
Corn	H.T.	2.75	0.17	0.25	0.16	1625
	L.T.	2.54	0.18	0.15	0.12	645
Bean	H.T.	1.86	0.17	0.18	0.25	1175
	L.T.	1.42	0.13	0.15	0.03	425

PERMEABILITY

It has been known for many years that light in the visible and near-ultraviolet regions of the spectrum directly alters the capacity of many plant tissues to take up inorganic salts and organic solutes through processes other than those involving transpiration. Likewise, the capacity to retain these solutes against osmotic gradients is influenced. Lepeschkin (*29, 30*) found that certain dyes are accumulated more rapidly in Elodea in light of relatively high intensities than in darkness. He concluded that the protoplasmic permeability was increased and that the most effective spectral region was in the blue and near-ultraviolet range from 320 to 420 mμ. Similar results were obtained by Offord and d'Urbal (*42*) with Nitella. Jacques (*23*) observed that the uptake of ammonium ions from sea water as well as the exosmosis of ammonium ions to ammonium-free sea water is higher in Valonia plants exposed to daylight than those kept in the dark. On the other hand, Zycha (*60*) and Jacques and Osterhout (*24*) have found that the accumulation of potassium in Nitella was relatively independent of radiation.

TABLE II

Influence of Transpiration on Absorption of Salt by Barley Plants of Low-Salt, High-Sugar, or High-Salt, Low-Sugar Status (from T. C. Broyer and D. R. Hoagland)

Experimental Conditions	Water Absorbed, ml./g. fresh wt.	Salt Absorbed in m.e. $\times 10^2$/g. total fresh wt. (loss from culture)		Total Sugar, g./l.	
	Shoot	K	Br	Shoot	Root
High salt; low humidity, light	8.10	5.20	6.07	5.4	1.1
High salt; high humidity, light	2.58	3.24	4.24	2.9	0.7
High salt; high humidity, dark	1.49	1.59	2.15	0.3	trace
Low salt; low humidity, light	9.60	10.85	9.52	15.3	3.5
Low salt; high humidity, light	3.60	10.40	9.65	6.2	2.5
Low salt; high humidity, dark	2.52	8.75	9.13	2.0	0.8

Recently, Lepeschkin (*31*) has reported that sunlight and ultraviolet radiation from a mercury arc accelerates the exosmosis of salts from the leaves of Sambucus and Parthenocissus and from potato tuber tissue. These results were obtained by measuring the changes in electrical conductivity of distilled water in contact with the excised leaves and potato tuber discs.

Hoagland, Hibbard, and Davis (*21*) and Lundegårdh (*33*) have shown that Nitella cells and excised roots, respectively, can carry out ion accumulation only in the presence of an adequate supply of oxidizable respiration substrates as carbohydrates. Thus, ion accumulation by green tissues is indirectly accelerated by light through photosynthesis. This factor seriously complicates the interpretation of experimental data obtained with green leaves. The fact that the shorter wave lengths of the visible and the near ultraviolet are the most effective spectral regions and that the effects are observable in nonchlorophyllous tissue, such as potato, would indicate that light appreciably alters the capacity of cells to exchange solutes other than by providing a source of carbohydrates for accumulation processes.

Stålfelt (*49*) and Virgin (*52*), using centrifugation methods, measured the change in viscosity in the leaves of *Helodea densa* in response to incandescent lamp irradiation. Virgin observed cyclic short- and long-term fluctuations, the pattern of which varied with light intensity; at intensities of 200 to 2000 foot-candles, a short transient increase in viscosity was observed followed by a rapid fall, while at the very low intensity of 0.05 foot-candle the viscosity decreased and remained low for several hours of continuous irradiation. The significance of these results in terms of the movement of solutes is not immediately apparent, but experiments of this nature should be borne in mind in evaluating the effect of light on solute movement and metabolism.

It is possible that light-induced permeability and viscosity changes may be the result of sensitized photochemical oxidations similar to those recently observed by Galston (*14, 15*) on the photooxidation of auxin and other cellular constituents with riboflavin, a fluorescing pigment of the respiratory enzyme systems, as the photosensitizer. Irradiation within the range of the principal absorption band of riboflavin, about 4600 A., results in the transfer of the energy to neighboring molecules which may then be oxidized in the presence of molecular oxygen. Several amino acids and indoleacetic acid have been shown by Galston to be readily photooxidized by riboflavin. One interesting feature about such a sensitized photooxidation is that the photosensitizing pigment is not itself rapidly destroyed in the process. It merely absorbs a quantum of light, becomes activated with excess energy, and transfers the energy to a neighboring molecule as activation energy or re-emits the energy as fluorescence, depending upon the opportunity for suitable collision. The activated neighboring molecule then uses the energy for reaction with molecular oxygen or degrades it to thermal energy. It is possible that riboflavin and other fluorescing pigments may carry out a wide variety of photochemical oxidations which may account for many of the rather obscure effects of light on plant cells such as light-modified permeability, viscosity, and protoplasmic streaming. The recent work of Goodwin and Kavanagh (*17*) on fluorescing substances in roots indicates that plant cells may contain a number of fluorescent substances capable of entering into such photooxidation systems.

It is impossible at the present time to evaluate the over-all physiological significance of light effects on permeability, photooxidations, and other such processes. Further investigations may show that these play a more significant part in the metabolic activities of the shoot than is now assigned to them. As far as roots growing in soil are concerned, however, these phenomena are obviously unimportant as directly controlling processes but they may exercise definite indirect effects.

PHOTOPERIODISM

Photoperiodism involves photochemical and thermal reactions initiated in the leaf which through the translocation of one or more hormones control the course of development of the terminal shoot meristems and in turn markedly influence the rate of shoot growth in many species. Higher plants fall into four classes as regards their flowering response to photoperiod on normal daily cycles: (*a*) long-day plants which flower on photoperiods longer than a certain critical day length, but remain vegetative on shorter photoperiods; (*b*) short-day plants which flower on photoperiods shorter than a certain critical day length; (*c*) intermediate plants which flower only on photoperiods within a narrow photoperiod range; and (*d*) day-neutral plants indifferent to photoperiod as regards flower bud initiation. In all these classes are plants whose flowering is also influenced by temperature. With some of these types in certain combinations of day and night temperatures, photoperiod has little influence on the course of development, the determination of the initiation of flower buds being controlled chiefly by temperature.

Numerous investigations have been conducted in an endeavor to correlate photoperiodic reactions with mineral nutrient supply and metabolism. With but few reported exceptions, variations in the mineral nutrient supply within the range capable of supporting growth have had no determinative effect capable of altering the course of floral initiation. The two environmental factors which appear to be determinative are relative length of the light and dark periods and temperature. In spite of the negative results obtained, however, several interesting correlations have been reported on the influence of mineral nutrient levels on the rate of appearance of floral primordia and the effect of photoperiod on the rate of appearance of nutrient deficiency symptoms.

Nitrogen relationships

Particular attention has been paid to nitrogen relationships. While the level of nitrogen appears to play no critical role in determining the induction of flowering, low levels of nitrogen do significantly affect the time of appearance of macroscopic flower buds. In short photoperiods, decreasing or removing the nitrogen supply decreases the rate of growth very markedly. In such cases, it has been found that the macroscopic flower buds of short-day plants often appear later than those of plants growing in a high nitrogen medium. In a long photoperiod such plants are vegetative at all nitrogen levels. This situation is exemplified in short-day plants, such as chrysanthemum (7), Xanthium (*39, 58*), soybean (*46, 58*), Kalanchoe (7), *Tinantia fugax* (7), *Setaria italica* (7), *Tithonia speciosa* (58), and *Salvia splendens* (58). For short-day plants in general, abundant nitrogen supply appears to hasten flowering. Without an adequate nitrogen supply, the number of floral buds set is sparse and fruiting often fails to occur.

Conversely, certain long-day plants in a long photoperiod favorable for flowering and deficient in nitrogen tend to develop macroscopic flower buds earlier than high nitrogen plants. This has been found to be true with barley (*3*), hard wheat (*7*), Iberis (*7*), spinach (*28, 58*), and lettuce (*7*). In a long photoperiod, the rate of growth is not decreased to the same extent by decreasing the nitrogen supply as it is in the case of a short photoperiod (*58*). In this same relation, Scully *et al.* (*45*) found that in onion, which is a long-day type of plant and forms bulbs only in a long day, a low nitrogen level tends to accelerate the rate of development of bulbing. These observations do not substantiate the oft-repeated horticultural concept that a low nitrogen level hastens maturity and the flowering of plants. This correlation appears to exist only for certain of the long-day and possibly the day-neutral types of plants.

Boron

The rate of appearance and severity of several of the micronutrient deficiency symptoms have been found to have a definite relation to photoperiod. Warington (*55*) observed that plants growing on a boron-deficient medium during the spring and fall months required a longer time for the development of the typical deficiency symptoms than similar plants grown during the summer. She investigated the possible relationships between temperature and day length to these observations and concluded that it was a photoperiod-controlled reaction. With broadbean, scarlet runner bean, Mandarin soybean, and barley, a nine-hour day very definitely retarded the appearance and progress of the boron deficiency symptoms as contrasted with a normal day length of over 15 hours. Biloxi soybean and garden pea exhibited no delay in appearance of symptoms in relation to photoperiod. The deficiency symptoms characteristic of lack of boron were similar regardless of time of appearance.

MacVicar and Struckmeyer (*34*) further demonstrated that Xanthium, tomato, sunflower, and buckwheat exhibited the usual boron deficiency symptoms in a boron-deficient medium during a 16-hour photoperiod. In a short photoperiod of nine hours, the deficiency symptoms did not appear in these plants. These experiments were conducted during the winter months when the daylight intensity was low and the photoperiod was short. Supplemental incandescent lamp irradiation was used to lengthen the photoperiod. Thus, the light intensity was relatively low as compared with summer conditions and the growth rate was correspondingly lower. These authors conclude that the lack of severity of boron deficiency symptoms under a short photoperiod is correlated with cessation of cambial activity and the occurrence of floral induction.

Manganese and iron

Steckel (*50*), working with soybean, has observed the appearance of deficiency symptoms for iron and manganese under conditions of different light intensities and day lengths, using varying ratios of iron and manganese. He found that the time of appearance of the symptoms could be correlated with light conditions. The symptoms appeared earlier at high light intensities than at low and earlier in long photoperiods than in short. Weight and top–root ratio data indicated that one of the chief factors probably responsible for the delay in appearance of the deficiency symptoms in short photoperiods and low intensities as compared with long photoperiods and high light intensities was a slower rate of growth.

CHLOROPHYLL SYNTHESIS

Chlorosis is one of the most conspicuous symptoms of both macronutrient and micronutrient deficiencies in higher plants and yet relatively little is known as to the biochemistry of chlorophyll synthesis and the mechanisms by which the lack of each element produces its own characteristic pattern of chlorophyll insufficiency. Nitrogen and magnesium are essential in the structure of the protochlorophyll and chlorophyll molecules. Galston (*13*) has reported that the chloroplasts contain from 30 to 40 per cent of the total nitrogen in both chlorotic and green leaves of oat. Granick (*18*) found similar results for tomato and tobacco, with 10 per cent of the chloroplast nitrogen present in the chlorophyll molecule. About 10 per cent of the magnesium in the plant is found in chlorophyll, with magnesium comprising about 2.7 per cent of the chlorophyll molecule. Several of the micronutrients, such as iron, zinc, and copper, appear to participate in certain of the enzymatic steps of chlorophyll synthesis. Phosphorus may very likely enter into energy exchanges associated with the synthesis. The role of the other elements in causing chlorosis is obscure.

In higher plants, chlorophyll synthesis is a photochemical reaction which proceeds at its maximum rate under relatively low light intensities. It appears to be a photocatalytic type of reaction in which the light energy is absorbed by a pigment which presumably is protochlorophyll. Protochlorophyll is present in the dark-grown leaf in relatively low concentrations as compared with the ultimate maximum concentration attained for chlorophyll in the green leaf (*47*). During the synthesis of chlorophyll, therefore, there must be a concomitant synthesis of protochlorophyll. The synthesis of protochlorophyll appears to be a thermal reaction and not directly dependent upon light. In the algae, such as Chlorella, the synthesis of chlorophyll can be thermally activated and will proceed in the dark in the presence of sugars. Both protochlorophyll and chlorophyll are magnesium porphyrins which may be extracted with ether and other lipoid solvents.

J. H. C. Smith (*48*) has recently initiated a program of study of chlorophyll synthesis and has redetermined the absorption spectrum of protochlorophyll. It contains two strong maxima which, in ether extracts, appear at 623 and 432 mμ, with the blue maximum much stronger than the red. There is appreciable absorption, however, throughout the ultraviolet and visible range from 250 to 650 mμ. The maxima coincide quite closely with the position of the action spectrum maxima of chlorophyll synthesis determined with light filters by S. Frank (*11*).

Smith (*48*) reported that, in the synthesis of chlorophyll, there is a concomitant decrease in protochlorophyll concentration and an increase in ether-soluble magnesium and phosphorus, the time-course patterns of which coincide closely with the appearance of chlorophyll. In the excised leaf, detached from the root system, the total ash, including magnesium and phosphorus, is relatively unchanged, but there is a large increase in ether-soluble fractions of both these elements. For leaves on an intact plant, however, there is an increase in ash corresponding with increases in lipoidal magnesium and phosphorus (Figure

1). Thus, there appears to be a mobilization of magnesium and phosphorus into leaves of greening barley seedlings and an increase in ether-soluble organic materials. Smith concludes that, in view of the relatively low concentration of protochlorophyll attained under equilibrium conditions in the dark, chlorophyll synthesis must be a rather complex series of reactions involving protochlorophyll synthesis with chlorophyll synthesis and the mobilization of magnesium and phosphorus into the synthesizing system. The significance of the accompanying increase of ether-extractable phosphorus is not clear, but phosphorylations may be involved. This increase is in harmony with Stoklasa's (51) original discovery that seedlings germinating in the light contain more ether-soluble and alcohol-soluble phosphorus than dark-grown plants.

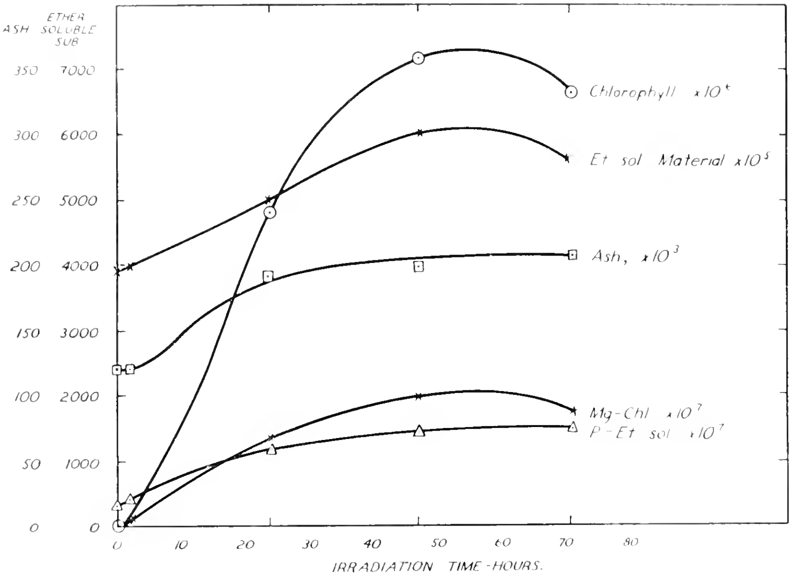

FIGURE 1. Effect of light on chlorophyll and other associated constituents of dark-grown barley leaves on intact plants.—J. H. C. Smith (47).

Composition given for chlorophyll, total ether-soluble material, chlorophyll magnesium, ether-soluble phosphorus, and total ash.

These results on the magnesium and phosphorus mobilization and conversion into ether-soluble compounds during chlorophyll synthesis may explain, in part at least, observations that magnesium and phosphorus are frequently taken up at parallel rates in higher plants.

Iron has long been recognized as an essential micronutrient for the synthesis of chlorophyll. Jacobson (25) determined the iron content of pear, tobacco, and corn leaves in relation to varying degrees of iron chlorosis. He found that both total iron and acid-soluble iron paralleled the chlorophyll content, but he found also that some of the iron is inactive in chlorophyll synthesis and this must be exceeded before chlorophyll formation can proceed. He observed that the "active" or ferrous iron was localized chiefly in the chloroplasts. This is in harmony with the idea advanced by Mommaerts (*36*) that chlorophyll in the leaf exists in combination with a protein and that such a chlorophyll-protein complex contains iron. In addition iron-containing compounds of this type have been isolated from corn and tobacco. It has been suggested that the iron is associated closely with a protein and that this protein complex, which apparently catalyzes chlorophyll formation, serves as a "chlorophyll enzyme."

Neish (*40*) has found that there is a greater percentage of copper in the chloroplasts than in the remainder of the leaf. In *Birfolium pratens,* the proportion was 74.6 per cent of the total. It has been found in some plants that there is a greater concentration and longer persistence of chlorophyll in copper-treated plants. Working with a variety of plants, Okunsov (*43*) found a marked increase in chlorophyll content of the leaves on the addition of copper sulfate spray when the plants were grown on a copper-deficient turf. The increased chlorophyll was held as principally due to a retarding effect of copper on the decomposition of chlorophyll with age. Arnon (*1*) has shown copper to be a component of the chloroplast of chard as a polyphenol oxidase. This is a copper-protein complex which may play a role in the evolution of oxygen by isolated chloroplasts during the water-splitting phase of photosynthesis.

PHOTOSYNTHESIS

Current interpretation of photosynthesis as an over-all process is tending increasingly toward the view that the reduction of carbon dioxide and the fission of water with the evolution of oxygen is a relatively complicated, many-step process closely integrated with the metabolic activities of the chlorophyllous cell. Of particular interest to the subject of this paper are evidences that phosphorus turnover and nitrate reduction are part of the photosynthetic process.

Before carbon dioxide enters the reduction phases of photosynthesis, it is fixed in the cytoplasm outside the chloroplast. The fixation is a nonphotochemical, enzymatic carboxylation in which organic acids are formed. This first step, known as carbon dioxide fixation, is not the characteristic feature of photosynthesis nor is it confined to photosynthesizing cells as it occurs in both autotrophic and heterotrophic organisms. After fixation, the carboxylated product is carried through the various stages of reduction; a hexose derivative usually appears as the end product.

The most characteristic feature of photosynthesis is the photochemical fission of water with the evolution of oxygen. It has been shown recently that isolated chloroplasts are capable of carrying out this stage of the reaction as a photochemical process. All the steps, from the initial fixation to the final fission of water and reduction of carbon dioxide, involve energy transfers which must require the storage of energy for very short periods in an available form in order to prevent back reactions and allow time for the next steps.

Phosphorus

The animal biochemists have shown that the energy transformation in the conversion of chemical energy to mechanical energy in muscle and the conversion of chemical energy from one form to another in the processes of respiration occur through the agency of phosphorylated compounds containing high-energy phosphate bonds. Several investigators have presumed that some of the energy storage processes of photosynthesis may involve similar energy transformations, postulating that inorganic phosphorus would flow into the photosynthesizing system when energy is stored but not used, and be released during energy utilization phases.

The downgrade flow of energy through respiratory processes has been shown to be carried out in plants, animals, and microorganisms by way of the high-energy phosphate bonds occurring in phosphorylated compounds such as adenosine triphosphate. Kalckar (*26*) and Lipmann (*32*) have very thoroughly reviewed the biochemistry of this subject. The role of phosphorus in the upgrade flow of energy in such organisms as the chemautotrophic bacteria which reduce carbon dioxide by the oxidation of reduced compounds as sulfur and hydrogen and in the photosynthetic organisms has been investigated only recently.

Vogler and his co-workers (*53*) have studied the exchange of phosphorus between cells of *Thiobacillus thiooxidans* and their environment in relation to the oxidation of sulfur and the utilization of carbon dioxide. This organism is a sulfur bacterium which synthesizes the materials needed for cell growth by the fixation and reduction of carbon dioxide with energy derived from sulfur oxidation. In the absence of carbon dioxide, Vogler found that sulfur can be oxidized and the energy stored within the cell and used later for the fixation of carbon dioxide under conditions unfavorable for sulfur oxidation. It was not determined experimentally whether the energy stored in this case could also be used for the reduction of carbon dioxide. It was suggested that, during sulfur oxidation in the absence of carbon dioxide, some of the energy is stored as phosphate bond energy and later used for carbon dioxide fixation with the release of inorganic phosphate.

During sulfur oxidation it was observed that, in the absence of carbon dioxide, inorganic phosphate was taken up from the external medium. Subsequently, in the absence of sulfur oxidation and in the presence of carbon dioxide, some phosphate was released to the external medium, presumably by the breakdown of energy-rich phosphate compounds synthesized during the sulfur oxidation phase. However, during normal sulfur oxidation in the presence of an adequate supply of carbon dioxide, no appreciable exchange of phosphate occurred, as phosphorylation and dephosphorylation presumably were in equilibrium. Adenosine triphosphate was identified as one of the phosphate compounds formed during the oxidation of sulfur and is possibly the phosphorylated storage product. Vogler suggested that in photosynthesis, energy from light absorption may be stored in a similar manner in high-energy phosphate bonds of phosphorylated compounds and later released for the fixation of carbon dioxide.

Ruben (*44*) measured the free energy of carbon dioxide fixation in

Chlorella and found that it was a very rapid reaction, even at low partial pressures of atmospheric carbon dioxide, indicating a low free energy per mol of about −2 kilogram-calories. The free energy of reaction of known carboxylations involving aliphatic and aromatic compounds is very much higher, being of the order of 10 kilogram-calories. Ruben postulated that high-energy phosphate bonds which have a free energy of 8 to 12 kilogram-calories per bond might be involved in this reaction.

Emerson, Stauffer, and Umbreit (*10*) and Ochoa (*41*) have postulated that the later steps following fixation of the carbon dioxide and involving the transfer of energy of the absorbed light quanta probably also occur through the agency of high-energy phosphate bonds. In endeavoring to test these hypotheses, Aronoff and Calvin (*2*) were unable to show any significant correlation between phosphate turnover and photosynthesis in Chlorella using P^{32} as a tracer. Gest and Kamen (*16*), however, also used P^{32} in Scenedesmus and Chlorella and found that such turnover studies could be carried out with consistent results only when low phosphate cells were used. In such cells, the soluble phosphate, readily exchangeable during washing processes, is reduced to a minimum and errors resulting from such manipulations are considerably reduced. They found that phosphate turnover in this case could be correlated directly with photosynthetic activity.

In Figure 2 are plotted the effects of light intensity on the trichloracetic acid-insoluble phosphate in Scenedesmus. The cells were grown on a low phosphate medium and the trichloracetic acid-insoluble fraction was found to be relatively stable as far as leaching was concerned. These data show that light markedly accelerates the formation of insoluble phosphate by the cells. Cyanide in Chlorella depressed the phosphate uptake; since the "ground respiration" of plant cells is cyanide resistant, these results indicate that the accelerated uptake is not correlated with respiratory activity that results directly from increased photosynthates. These experiments indicate that ester phosphate may be formed as a result of light absorption, but the evidence is insufficient to determine whether such esterification is directly coupled with the reactions of photosynthesis.

Wassink *et al.* (*56, 57*) have obtained somewhat similar results with photosynthesis in the purple sulfur bacterium, *Chromatium*, strain D. Irradiation caused a marked uptake of phosphate in an atmosphere of nitrogen and oxygen. When carbon dioxide was present, the uptake was less, indicating the possible utilization of ester phosphate in carbon dioxide reduction. When the cells were transferred to darkness, there was a small release of inorganic phosphate, apparently associated with metabolic energy release.

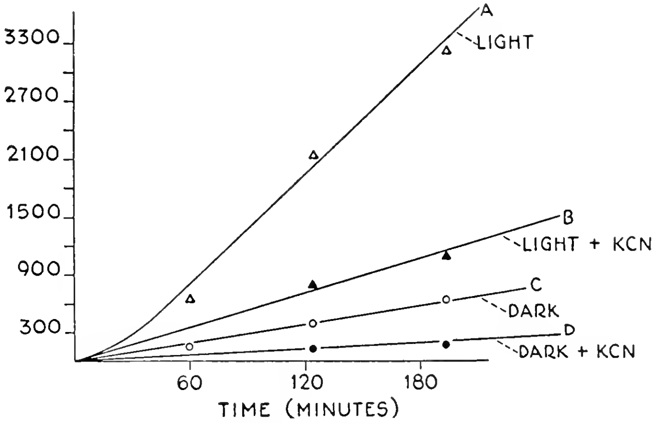

FIGURE 2. Effect of light on P uptake by Scenedesmus D_3.—Gest and Kamen (16).
Data are for trichloroacetic acid-insoluble cellular residue.

These few rather recent investigations strongly suggest that one of the most important roles of phosphorus in plants may be in phosphorylation reactions involved in the upgrade flow of energy in photosynthesis.

Nitrogen

The two principal sources of inorganic nitrogen for the growth of higher plants are ammonium and nitrate. Ammonium nitrogen is already in a reduced form and in the dark is readily assimilated into organic forms of nitrogen. Nitrate nitrogen, on the other hand, must be reduced at least as far as nitrite before it can be assimilated into organic nitrogen. In the roots of most woody plants and legumes, this process takes place as a purely thermal reaction in the dark. Since the reaction is exergonic, energy is required which, in the case of nitrate reduction in roots, is derived from respiration.

In 1920, Warburg and Negelein (*54*) observed a low assimilatory quotient with Chlorella which they assumed was due to the reduction of nitrates by some photosynthetic process. Burström (5, *6*) later extensively studied nitrate reduction in the wheat plant. He found that the root was capable only of thermal reduction, which was independent of light, but required an external supply of manganese. Kinetical studies indicated that the reduction occurred at the very outer surface of the root cells and that, while manganese was essential, iron could partially substitute for manganese.

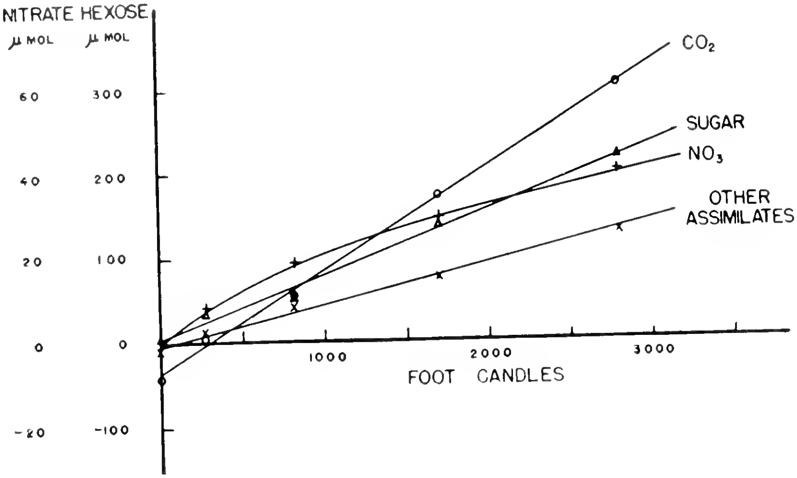

FIGURE 3. Nitrate reduction in excised wheat leaves as a function of photosynthetic rate over a 24-hour period in an atmosphere of about 10 per cent higher than normal air carbon dioxide.—H. Burström (5).

Data are for apparent carbon dioxide assimilation, sugar formed, nitrate lost from leaf tissue, and other assimilates (CN compounds) formed.

In contrast to wheat roots, the leaves were completely unable to carry out nitrate reduction in the dark. In the presence of light and carbon dioxide, however, nitrate reduction occurred and the rate paralleled very closely the photosynthetic rate as shown in Figure 3. This process does not appear to be the result of the synthesis of carbohydrates as such, since it will be noted that even when the light intensity drops below the compensation point and respiration is consuming more carbohydrates than are being formed, nitrate reduction still proceeds. Neither does the process appear to be a simple photochemical reduction of nitrate as a substitute for carbonate since carbon dioxide is likewise essential as exhibited in Figure 4, where nitrate reduction was carried out in an atmosphere containing only about 10 per cent normal atmospheric carbon dioxide concentration. When the carbon dioxide concentration is very low, nitrate reduction appears to take precedence over sugar formation; possibly some intermediate product of carbon dioxide reduction is utilized in the reduction of nitrate, thus blocking sugar formation. At high light intensities, sugar formation practically ceases in a low carbon dioxide atmosphere. These experiments at low carbon

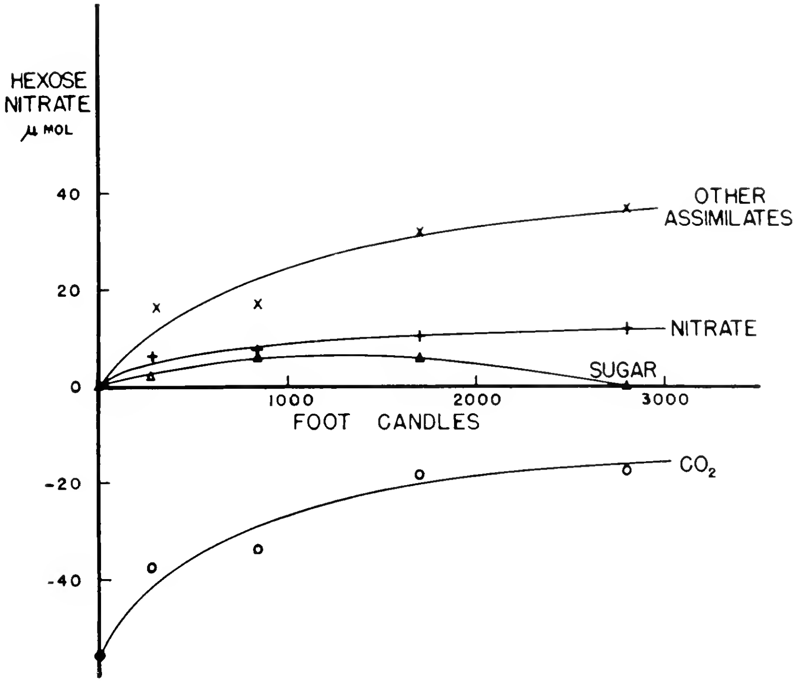

FIGURE 4. Nitrate reduction in excised wheat leaves in a low carbon dioxide atmosphere, about 10 per cent of normal air.—H. Burström (5).

dioxide concentrations show that about 15 mols of carbon dioxide are used in the reduction of one mol of nitrate. Thus it appears that in the process of reduction of nitrate and carbon dioxide, some nitrogenous organic compound is formed.

Myers (37, 38) and Cramer and Myers (8) have studied nitrate reduction in Chlorella. This alga behaves like both the root and the leaf of wheat in that it is capable of both thermal and photochemical reduction of nitrate. The data in Table III show that the assimilatory quotient (CO_2/O_2) is considerably less than one (0.68) in the presence of nitrate. In the presence of a mixture of nitrate and ammonium or ammonium alone, the assimilatory quotient is nearly one (0.94). When ammonium is present, photochemical nitrate reduction ceases.

TABLE III

CO_2/O_2 Quotient for *Chlorella* Cells Grown at 40 Foot-candles and Studied at 40 Foot-candles (from Jack Myers, ref. 38)

Nitrogen Source	CO_2/O_2 by Manometric Measurement	CO_2/O_2 Calculated from Cell Analysis
NO_3^-	−0.68	−0.69
NH_4^+	−0.94	−0.91
$NO_3^- + NH_4^+$	−0.94	—

Some have objected to any concept which requires two such diverse pathways for nitrate reduction. This does not, however, appear to be a serious factor considering that in carbon dioxide reduction, two pathways have already been shown to exist in some of the lower organisms, one thermal and one photochemical. In the autotrophic chemosynthetic organisms, energy is derived from the oxidation of sulfur or hydrogen, which in turn is used for the fixation and reduction of carbon dioxide. In the presence of light, however, some of these organisms carry out a photosynthetic reduction instead of a thermal reduction.

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CHAPTER

17

Soil Moisture and the Mineral Nutrition of Plants

C. H. WADLEIGH and L. A. RICHARDS

THE MINERAL NUTRITION of plants is related in many ways, directly and indirectly, to soil moisture. It is the purpose of this paper to discuss some of these relations and it seems appropriate to introduce the subject with a review of some of the modes of behavior of water in soil. Particular attention will be given to the physical condition of moisture as it relates to the readiness with which roots may absorb moisture present at the soil-root interface, and also the readiness with which additional moisture at some distance from the roots can flow toward the roots to replace that which is absorbed.

PHYSICAL CONDITION OF MOISTURE IN SOIL

Soil particles are wetted by and have considerable attraction for water and, therefore, soils exhibit a capacity to take up and retain moisture. This moisture is distributed throughout the pore system of the soil and over the surface of the soil particles. Energy is involved in this adsorption because work is required to remove moisture from soil. The amount of work per unit amount of water removed will depend on the moisture content of the soil. This work can be expressed in terms of the pressure difference through which a unit volume of water must be transferred to effect removal of water from the soil. In saturated soil the pressure in the soil water approaches zero at atmospheric pressure. Water drops will form and drip from soil which is very wet, so the work that must be done against surface force action in order to effect water removal from very wet soil approaches zero. As soil dries out, more work must be done to extract water, and the equivalent negative pressure in the soil water increases. This pressure, as far as soil moisture energy relations are concerned, is equivalent to tensile stress and is referred to as soil moisture tension.

Other factors being favorable, plants can grow and absorb moisture at soil moisture contents ranging from saturation down to some minimum moisture content that is associated with the wilting of plants and which depends on the texture of the soil. As this minimum moisture content is approached and the plant loses turgor, the rate of growth of the plant approaches zero. The wilting percentage may be as low as 2 or 3 per cent for sands or as high as 30 or 40 per cent for clay and peat soils. Normally, in the root zone in the field, soils do not become completely saturated. The soil moisture tension actually drops to zero only in the shallow surface layer. After heavy rain or irrigation, the water contained in the large pores of the soil drains away freely under the influence of gravity. This drainage rate is rapid at first, but after two or three days, in soils that do not have restricted drainage or a water table, the rate of drainage becomes negligible. The moisture content of the soil, after drainage becomes negligibly slow, is designated as the "field capacity." Consequently, it is common to refer to the moisture content of soil from field capacity to the wilting percentage as the "available range," or the range of moisture that is available for producing plant growth.

The equivalent negative pressure in the soil ranges from zero in saturated soil to something of the order of 0.05 to 0.15 atmosphere at field capacity and on up to about 15 or 20 atmospheres at the wilting percentage for crop plants. This pressure scale is convenient since it is a direct measure of the work that plants must do to absorb water against surface force action in the soil. The relation between the moisture tension and the moisture content of soil is hyperbolic in nature (*103*) as illustrated by the curves in Figure 1. It is seen that a considerable amount of water may be withdrawn from wet soil before the tension rises more than an atmosphere or two. However, at tensions above 8 or 10 atmospheres a small decrease in moisture content corresponds to a large increase in soil moisture tension. The curves in Figure 1 illustrate typical moisture-tension relations over the whole plant growth moisture range and for a wide range of soil textures.

The combination of a porous cup and a vacuum gage, which has been called a tensiometer, can be used for measuring soil moisture tension over the range from zero to about 0.85 atmosphere. The relation of soil moisture content to soil moisture tension over the whole plant growth range has been studied by means of porous membrane apparatus (*102*). Recent improvements have been made in measuring methods so that it is now conveniently possible, by the freezing point procedure, to estimate soil moisture tension in nonsaline soils from freezing point depression values (*104*). Soil moisture tension is thus an experimentally usable scale for expressing the security or tenacity with which water is held in the soil by surface force action. If appreciable quantities of soluble salt are present in the soil, then an osmotic force also resists the absorption of water by plants and must also be taken into consideration. The sum of the soil moisture tension and the

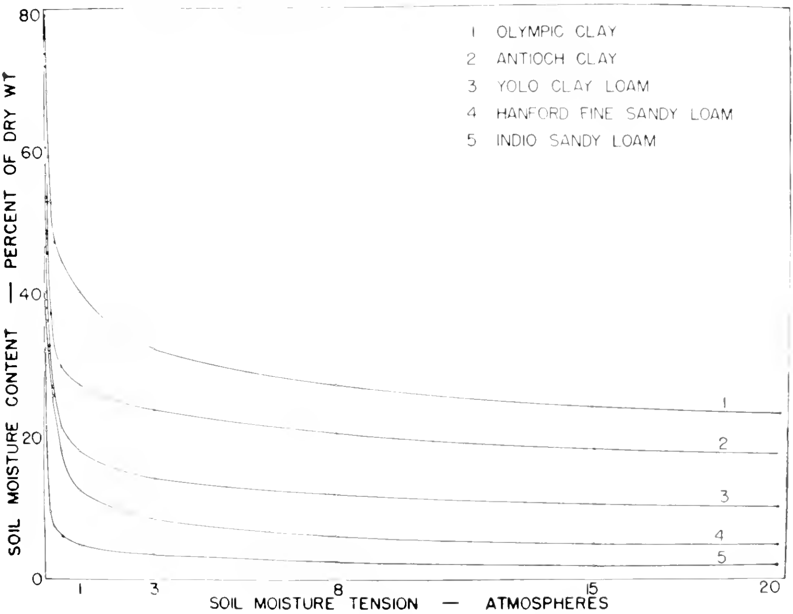

FIGURE 1. Curves showing the relation between the soil moisture tension and the moisture content of the soil.

osmotic pressure of the soil solution has been called the total soil moisture stress (*128*) and can be used as a measure of the total amount of work that a plant must do to absorb a unit amount of water from the soil. The freezing point measurement directly gives an indication of the total soil moisture stress.

Furr and Reeve (*46*) have shown that the rate of growth of sunflower plants drops to zero as the soil moisture approaches the wilting percentage. There is increasing experimental evidence that the growth of crop plants ceases when the soil moisture stress rises into the range of 15 to 20 atmospheres (*130*).

The course of events during moisture depletion in field soil may be set forth briefly by an illustration. Consider a newly planted hill of corn after a thorough soaking rain. When the rain stops, the moisture moves gradually out of the larger pores toward the water table which, for best crop growth, should be below the root zone. This water movement takes place primarily under the influence of gravitational force because, when the whole profile is very wet, gradients in soil moisture tension are small.

Concomitant with the recession of water in the larger pores, air is drawn into these pores. As the soil approaches field capacity there is a reasonably continuous gaseous phase throughout the soil, but the moisture films covering the soil particles become so thin that additional moisture movement in these films is considerably restricted.*

Thus, several days after the rain has stopped, the soil moisture tension may rise into the range of 0.05 to 0.15 atmosphere (50 to 150 cm. of water) and moisture movement in the profile becomes considerably restricted. Water vapor loss will occur from the top few inches of surface soil due to air circulation and the elevated temperature of the surface soil, but moisture loss from soil layers a few inches below the surface is usually slow. When the corn seeds germinate and the plant roots develop in the neighborhood of the kernel, the rootlets remove moisture from the immediately adjacent soil and can build up soil

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* It should be kept in mind that the condition here referred to as field capacity can exist only in well-drained soils. When soil horizons having low permeability are present in the profile, or when a nearby ground water table exists, then the root zone may remain excessively wet for long periods.

moisture tensions of the order of 10 to 20 atmospheres. The high tension in the soil moisture in the vicinity of the root sets up a force action in the soil-water system that tends to move water toward the root. This tendency of water to move toward plant roots in response to tension gradients must be of considerable importance, particularly for perennial plants with large developed root systems, because a small distance of movement over a considerable combined length of root system would account for an appreciable volume of water. However, for young plants with a newly developing root system, this movement is so slow that sufficient water for normal growth would not be supplied unless the plant roots are able to extend themselves outward into a fresh soil moisture supply. Therefore, as has been described by Davis (*35*), when a new corn plant is developing, the available moisture is extracted in the vicinity of the base of the plant and the soil approaches the wilting condition whereas just a few inches further away from the plant the soil may be at or near field capacity. The roots of the newly developing plant must extend themselves outward in order to maintain a continuous supply of available water. When the roots of the plant have permeated the soil region in which they can grow well, the soil moisture tension throughout the soil region occupied by roots will approach that corresponding to the wilting percentage, and, unless additional moisture is supplied by rain or irrigation, vegetative growth will cease and the plant will show symptoms of wilting. The depletion of soil moisture under perennial plants with developed root systems has been described in detail by Veihmeyer and Hendrickson (*123*).

During this process of moisture extraction by roots the soil water is withdrawn successively from the large, medium, and fine pores and is replaced by a gaseous phase. Thus, aeration processes are expedited and become freer as the moisture content of the soil is depleted. Soils having favorable structure for the growth of plants tend to have an appreciable fraction of the pore system made up of large pores. These pores are the first to empty during moisture depletion, thus promoting good drainage and favorable aeration.

From saturation to the field capacity, moisture depletion is accounted for by downward drainage. Leaching and loss of soluble material from the profile occurs during this process. After the field-capacity condition is attained, however, moisture depletion is largely brought about by plant root extraction. In this case the soluble materials in the soil solution are either absorbed by the plant during moisture uptake or, as is the case in saline soils, the soluble salts are largely left in the soil because of the selective absorption of the plant root. Moisture depletion is thus accompanied by a considerable change in the concentration of the soil solution. This change in the concentration of the soil solution brings about concurrent changes in the equilibrium between the adsorbed and the soluble ion phases.

It was indicated earlier that the freezing point depression gives a measure of the total soil moisture stress. The aqueous vapor pressure of the soil water is another measure of the free energy of the soil water or the total soil moisture stress. In the plant growth moisture range, the pressure of the water vapor in the soil atmosphere is always very high and near the saturation pressure at the particular temperature. The relative humidity range from 98 to 100 per cent more than covers the available moisture range in all soils. In other words, the relative humidity in soil from which plant roots are extracting water for growth never goes below about 98 per cent.

MOVEMENT OF MOISTURE IN SOIL

The movement of water into and through soil can be conveniently expressed in terms of the force which tends to produce the motion of the water. Gravity and the gradient of the moisture tension in the soil water are the two components that must be considered. Gravity always acts in the downward direction. The component of force arising from the tension gradient in the soil water, however, may act in any direction. When water is at rest under gravity and hence is at static equilibrium, the pressure gradient force is equal to and opposite to gravity. The pressure gradient force in soil moisture can be conveniently estimated from tensiometer readings and these instruments cover the tension range in which ready movement of soil moisture takes place. The vector sum of the gravity force and the pressure gradient force can be most conveniently expressed in terms of the hydraulic gradient. When the pressure force vanishes, i.e., when the soil moisture tension of the soil water is everywhere the same, then the net water moving force is simply and entirely gravity force, i.e., "one g," and corresponds to unit hydraulic gradient. Permeability tests on the rate of movement of water in soil cores or soil samples in the laboratory are often conducted at unit hydraulic gradient. When a thin covering layer of water is maintained on the top of the soil column and the water outflow at the bottom of the soil column is at atmospheric pressure, there is no pressure gradient in the soil column and gravity is the only force acting. The velocity of water in soil at unit hydraulic gradient is designated as permeability and is a measure of the readiness with which a soil transmits water.

Darcy's law for the movement of water in soils simply states that the velocity of water movement (v) is proportional to the hydraulic gradient (i) as expressed by the equation $v = Pi$. The proportionality constant, P, is the permeability.

The hydraulic head at any point in a soil moisture system is equal to the elevation at which water will stand in a riser or piezometer tube connected to the point in question (*101*). If the soil is not saturated, a porous cup can be used for establishing connection between water in a manometer and water in the soil, as shown in Figure 2. In practice, mercury manometers are used so as to allow measurements to be made above ground, but actually, water manometers such as those illustrated at the right in the figure could be used. When the porous cup is in unsaturated soil, the free surface of the water in the manometer will come to rest at a level below the porous cup. The difference in level between the cup and the free water surface is a direct measure of the soil moisture tension at the porous cup, whereas the elevation of the free surface referred to any convenient datum is the hydraulic head of the soil water at the cup. The average hydraulic gradient between two points on a flow line, i.e., the soil moisture tension gradient plus the component of gravity force, is equal to the difference in hydraulic head between the points divided by the distance along the flow line. These relations can be illustrated by the tensiometer system shown in Figure 2.

Let it be assumed that the manometer readings represent moisture conditions a week after a rain on a fallow soil and, for simplicity, let it further be assumed that moisture conditions do not change in the horizontal direction. The moisture tension at the depth of a porous cup, when expressed in terms of a length of water column, is equal to the vertical distance from the cup down to the free surface of the water in the manometer. This tension can be read directly from a scale on the mercury manometer (*102*). Tension readings measure the tenacity with which water is held by soil, but are not simply related to moisture movement because gravity force is not taken into account. Hydraulic head and hydraulic gradient on the other hand do take gravity into account.

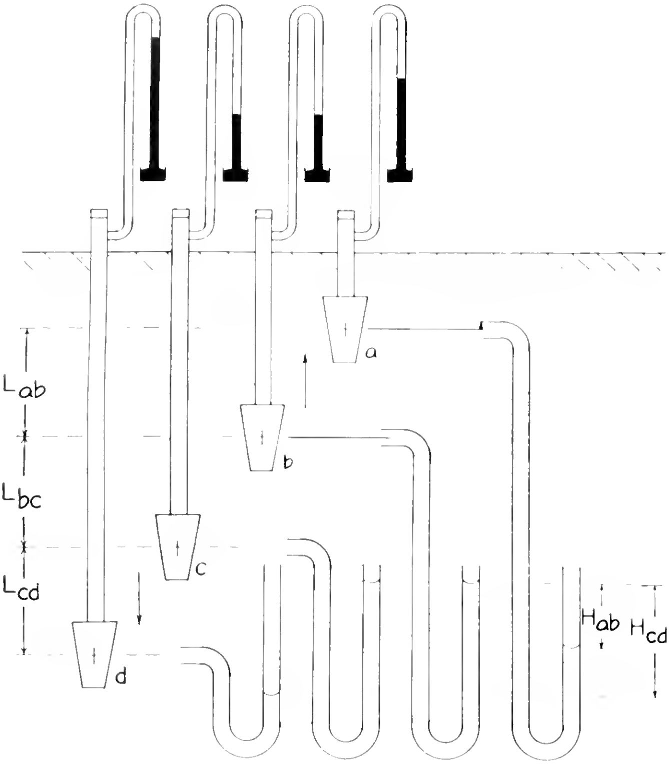

FIGURE 2. Porous cup-manometer system for measuring the hydraulic head and hydraulic gradient of water in unsaturated soil. *See page 417.*

As defined above, the hydraulic head of the water in the soil adjacent to a porous cup is equal to the elevation of the free water surface in the corresponding water manometer. In a connected soil moisture system, moisture always streams through soil in the direction of the decrease in hydraulic head. It is apparent that the hydraulic head at cup b is higher than at cup a. Therefore, water is moving upward in the soil layer a—b as indicated by the arrow.

In a soil region where the hydraulic head is everywhere the same, water will be at rest under gravity. The tension gradient will be of such size and direction that it will just cancel gravity. The hydraulic head at cups b and c is the same, so that on the average the hydraulic gradient in this interval is zero. The hydraulic head at cup c is higher than at cup d, so that in the interval c—d water is moving downward, as indicated by the arrow.

The hydraulic gradient is the change in head per unit distance along a flow line. So in the interval a—b we have for the hydraulic gradient $i_{ab} = H_{ab}/L_{ab}$. In the soil interval b—c the average hydraulic gradient is zero, because H_{bc} is zero. This should not be interpreted to mean that the transmission velocity at b and at c is zero, but rather that there is a soil layer between a and b above which the moisture is moving up and below which the moisture is moving down. In the lower soil interval, $i_{cd} = H_{cd}/L_{cd}$ and is in the opposite direction to i_{ab}. Thus, the direction in which water is streaming through soil can be predicted from hydraulic head measurements.

Darcy's law for water movement in soil has long been recognized to hold for saturated soils. Its application to unsaturated soils is not so familiar even though the definition of hydraulic head and hydraulic gradient for the unsaturated case as just given are substantially identical to the saturated case. The only difference lies in the fact that in unsaturated soils some of the pores are filled with gas and are not available for transmitting water.

As the soil dries out and the pore spaces become filled with air, moisture movement must take place in the water films over the surface of the soil particles. Thus, the permeability changes with the moisture content of the soil. For example, it is found that for a sandy soil which is saturated with water, the soil transmits 8 cc. of water per square centimeter per hour at unit hydraulic gradient. The permeability is thus 8 cm. per hour. At a soil moisture tension of 100 cm. of water, which for this soil corresponds approximately to the condition of field capacity, the permeability is about 0.2 cm. per hour or 1/40 of the value at saturation. At a tension of 200 cm. or approximately 0.2 atmosphere, the permeability drops to 0.0013 cm. per hour which is 1/6000 of the value at saturation. Measurements of this type obtained a number of

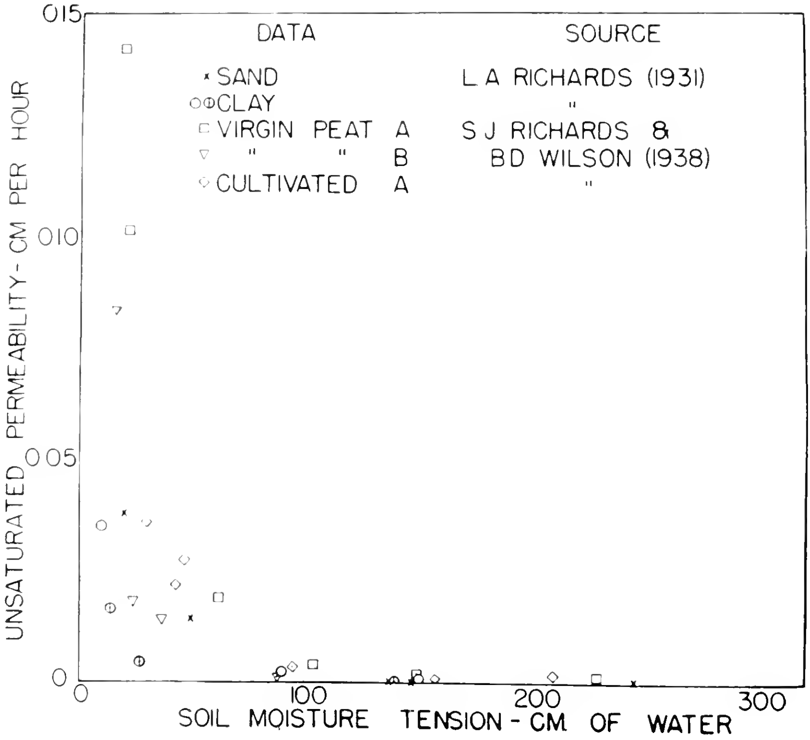

FIGURE 3. Relation of unsaturated permeability of soil to soil moisture tension. The data points illustrate the rapid decrease in the permeability with a small increase in tension.

years ago by L. A. Richards for mineral soils (*100*) and by B. D. Wilson and S. J. Richards (*135*) for peat soils are plotted to a linear scale in Figure 3. At saturation, the permeability of many normal soils will lie in the range from 1–10 cm. per hour. This range is far above the values shown for unsaturated soils, even at the low tensions of 10–20 cm. of water. It is evident that as air replaces water in the soil pores and the soil moisture tension increases, there is a very rapid decrease in the readiness with which the soil transmits water. Two or three days after a heavy rain or irrigation, tensiometers in field soils usually read in the range from 50–150 cm. of water. It thus appears that field capacity corresponds to the moisture content at which the moisture films are so thin that the unsaturated permeability becomes very small and, compared with plant root extraction, further downward drainage can be neglected.

It is of interest to consider some soil moisture systems that are related to plant nutrition and experimental plant growth setups. Take for example the small pots in which plants are frequently grown. When the soil in a pot is allowed to come to equilibrium with a water table at the bottom of the pot, we have the condition that the soil moisture tension is zero at the water table. When equilibrium is established the soil moisture tension at each level in the pot is just equal to the vertical distance to the water table. Near the water table the tension is low and the moisture content of the soil is correspondingly high. All the soil pores, except the very largest, will thus be filled with water. As the distance from the water table increases, the larger pores are increasingly filled with air, the soil moisture tension is higher and the moisture films on the soil particles are thinner. This soil, pore-space, moisture system has been nicely pictured by Gardner and Chatelain (*47*). For a soil water system that is at equilibrium with the water table and which is protected from evaporation, the hydraulic head of the water in the system is uniform throughout; the hydraulic gradient and hence the water-moving force in the system are balanced because the downward force of gravity is just counterbalanced by the increase in tension with height. If evaporation is allowed to take place from the soil surface, this increases the tension gradient over and above that required to overcome gravity and an upward flow takes place. When plants are grown in pots, it is interesting to note that the soil moisture conditions at the bottom of the pot are very similar to those just described. No outflow of water will take place until the soil moisture tension at the bottom of the pot becomes zero, thus downward drainage of water from a pot takes place just as if there were a water table at the bottom of the pot. Consequently, the soil moisture that exists in a shallow pot when an excess of moisture is applied and drainage is allowed to take place is at a considerably higher level than that which would exist in this same soil in the field. This higher moisture content may considerably alter the aeration and the physiological status of the plants, especially in fine-textured soils.

When the moisture content of the soil is at field capacity or above, small changes in the hydraulic head can bring about corresponding movement in the water in the soil. However, when the moisture content of the soil is below field capacity, which corresponds to soil moisture tensions of the order of 50–150 cm. of water, then the unsaturated permeability of the soil is small, and moisture movement, i.e., the velocity of movement, is restricted even for large hydraulic gradients.

When the moisture in soil has become reduced to near the wilting percentage by plant root extraction, it is found that the process of rewetting of the soil follows a fairly regular pattern. In order to get water to move into and through the soil, enough water must be added to the wetted zone to raise the moisture content of that zone above the field capacity. It must be accepted as an experimental fact that there is a comparatively steep moisture gradient in the wetting front. In order for wetting to proceed, the moisture content of the wetting front must be raised to field capacity or above, otherwise the permeability of the soil will be too low to transmit moisture at a rate that will allow the wetting process to proceed at an appreciable rate. When a limited amount of water is added to dry soil, the moisture content of the wetted portion is raised to field capacity and the rest of the soil will remain dry. This phenomenon has been discussed by Shaw (*108*), Veihmeyer (*120*), Colman and Bodman (*33*), Kirkham and Feng (*68*), and others. As a consequence of this wetting phenomenon, it appears that for practical purposes the only method for controlling soil moisture during plant growth is to limit the degree of dehydration before the whole root zone is brought to field capacity, or above, by irrigation. Nevertheless, papers continue to appear in the literature reporting experiments in which plants were grown at constant, controlled soil moisture values. The average moisture content for the whole soil volume may have been maintained nearly constant but there is considerable doubt in such experiments as to whether the moisture was evenly distributed after limited water applications.

INFLUENCE OF EXCESS MOISTURE ON NUTRIENT SUPPLY AND AVAILABILITY

The influence of an excess of water upon nutrient supply and availability in soils may be segregated into three categories, viz: (*a*) surface erosion, (*b*) leaching, and (*c*) presence of a high water table or other conditions maintaining soil in a relatively wet state. Virtually no soil is devoid of the occasional presence of one of these effects, and frequently two or all three of them may be operative.

Surface erosion

Agriculturists in this country have become increasingly aware of the vast losses in soil fertility incurred by surface runoff. This has been so well publicized that further mention seems superfluous. Yet the enormity of this loss mitigates any danger of overemphasis. Fippin (*42*) presents data to the effect that the Mississippi River carries 475 million tons of silt into the Gulf of Mexico during the average year, and that this silt load contains 4.5 million tons of the exchangeable bases—calcium oxide, magnesium oxide, and potassium oxide—and 1.5 million tons of phosphoric anhydride and nitrogen. This is indeed an enormous quantity of plant nutrients. Fippin also calculated that for the year 1939, the average loss of plant nutrients per acre of row crops in the Tennessee River system was "84.6 pounds of calcium, 97.9 pounds of magnesium, 212.2 pounds of potassium, 13.0 pounds of phosphorus, all expressed as oxides, and 23.8 pounds of nitrogen." These figures amply indicate that surface runoff may bring about an alarming drain on plant nutrients present in the relatively more fertile top soil. However, these values are much higher than those reported by Bryant and Slater (*23*) for two New York soils subjected to seven different kinds of cover. The small losses of nutrients which they found in surface runoff would probably be compensated by contributions from soil formation processes.

Leaching

Soil management practices inducing infiltration and curbing nutrient loss from surface runoff do not necessarily prevent nutrient loss by percolation. The objection might be raised that prevention of surface runoff merely alters the manner in which plant nutrients are removed from the soil but not the amount. It is certain that liquid water may not move through a soil without carrying solutes with it. A vast amount of data on leaching has been provided by lysimeter studies. Kohnke *et al.* (*71*) have provided an excellent bibliography and summary of the information available. Since soils have virtually no adsorptive capacity for nitrate and chloride ions, these two anions are readily leachable and the quantities of these ions lost by leaching are rather closely related to the quantities present in the soil and the amount of percolate. The sulfate and bicarbonate ions are also readily leachable, whereas phosphate is usually present in leachate in very small quantities, if at all.

Loss of nutrient cations by leaching will be conditioned by base exchange phenomena. Of the exchangeable bases usually found in soils, sodium has a relatively low energy of retention by adsorption surfaces and is most readily leachable. Consequently, it has been readily leached from humid soils, and therefore it is not prevalent in percolate from soils of humid regions in marked contrast to that from soils in arid regions. Potassium also has a relatively low energy of retention by soil colloids, but it may readily become fixed within the crystal lattice of soil clays. Consequently, it usually occurs in the leachate in rather minor quantities. Thus, calcium and magnesium are almost invariably the predominant cations in the leachate from lysimeters. As Kohnke *et al.* (*71*) point out, the actual quantity and quality of nutrient loss by leaching depends on many different factors. Thus, coarse-textured soils permit a greater proportional loss of nutrients than fine-textured soils, and a porous-crumb structure favors greater percolation than a single-grain structure. The type of soil cover may markedly affect nutrient loss by leaching. Thus, Lyon and Bizzell (*79*) found that an uncropped Dunkirk silty clay loam lost 28 times as much nitrogen by leaching as compared to the same soil continuously cropped to grass. There is much evidence to the effect that a vegetative cover lowers nutrient loss from leaching, by effecting a reduction both in the amount of leachate and in the content of nutrients in the leachate.

Dreibelbis (*38*) reported that drainage from elaborately designed monolith lysimeters containing a Keene silt loam showed an average annual loss of nutrients in pounds per acre as follows: calcium, 19.9;

magnesium, 10.3; potassium, 13.4; manganese, 0.3; nitrogen, 2.6; and sulfur, 17.8. Losses from a Muskinghum silt loam averaged lower for calcium, potassium, and sulfur but higher for nitrogen. Even though some data indicate that nutrient loss by leaching may be large, these observations do not warrant such a conclusion. Kohnke (*70*) has emphasized that losses of plant nutrients in drainage are appreciably less than those possible from surface runoff.

When fertilizer is applied to a soil and conditions conducive to leaching prevail, there is usually an increase in solute in the leachate, but the proportions of the component ions of the solute usually deviate from those in the added fertilizer due to base-exchange reactions. This is well illustrated by the observations of Volk and Bell (*126*) on lysimeters filled with Lakeland loamy fine sand having a base-exchange capacity of 3.0 m.e. per 100 g. Various sodium, magnesium, and potassium salts were applied to different lysimeters. Though no calcium was applied, it was invariably the predominant cation in the leachate. Even when 16.39 inches of leachate had been collected from the 4-foot columns of soil, only about 1 per cent of the potassium applied was found in the leachate, whereas about 65 per cent of applied sodium and about 30 per cent of applied magnesium appeared in the leachate. Chlorides were recovered almost quantitatively; about 50 per cent applied sulfate appeared in the leachate and, in most instances, more than twice as much nitrate was recovered as was applied. There was practically no difference in the proportions or amounts of cations in the leachate whether the salts added to the soil were chlorides or nitrates, but when sulfate salts were applied, there was a marked decrease in the loss of calcium.

The foregoing data emphasize that leaching losses of nutrients may be high on a light soil under heavy rainfall. Thus, the standard treatment of Volk and Bell (*126*) corresponded to an application of 31 pounds of nitrogen per acre, and the leachate from this treatment corresponded to a loss of 74 pounds of nitrogen per acre from fallow soil over a five-month period. It is commonly observed that nitrogen deficiency may readily develop on light soils during wet seasons. There is increasing evidence that these same conditions are conducive to the development of magnesium deficiency. Hester *et al.* (59) noted that magnesium deficiency symptoms were quite prevalent on sweet potatoes and tomatoes growing in sandy soils in New Jersey during the relatively wet season of 1946. They state that extreme magnesium deficiency is not likely to occur on this soil except under conditions of high rainfall or heavy potash fertilization. Boynton *et al.* (*16, 17*) have noted that magnesium deficiency symptoms are more prevalent in the apple orchards of New York during wet years than dry.

The preponderance of evidence indicates that percolation of water through a soil tends to effect the depletion of sodium, calcium, and magnesium ions with relatively little removal of potassium. Jamison (*65*) reports that potassium is readily leached out of certain sandy soils in Florida in which the exchange capacity is largely provided by organic matter because of the inability of organic adsorbents to fix potassium.

It is probable that in most instances losses of nutrients by leaching do not exceed contributions from soil decomposition and nitrogen fixation over a long period of time.*

High water tables and wet soils.

It is evident that under certain conditions serious losses of plant nutrients from the soil may arise from either surface runoff or downward percolation. In addition, retention of excess soil moisture within the root zone may have a seriously detrimental effect on the nutrition and health of crop plants. Impervious layers in the subsoil that bring about permanent or even temporary water tables affect soil aeration, and, consequently, root growth, activity of microflora, nutrient availability, and nutrient entry. As mentioned previously, soils with low permeability may also provide adverse conditions within the rhizosphere following a heavy rain or after an irrigation. As the soil moisture tension becomes lower, the soil pores become increasingly filled with moisture and gaseous interchange is inhibited. Observations on the composition of soil air illustrate the result of this relationship. Furr and Aldrich (*45*) studied the composition of the soil atmosphere at various depths under irrigated date palms. On irrigating, the soil

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* Since the completion of this manuscript, a pertinent study by Chapman *et al.* on nitrogen gains and losses in lysimeters has come to the attention of the authors. *See* H. D. Chapman, G. F. Liebig, and D. S. Rayner, *Hilgardia,* 19:57 (1949).

moisture tension at the six-inch depth dropped from 700–800 cm. of water to 11–15 cm. and remained that low for 4 or 5 days after irrigating. During this period of low moisture tension, oxygen content of the soil atmosphere dropped from about 20 per cent to 5 per cent. However, a concomitant change in carbon dioxide content was not noted. As soon as 100 cm. of water tension developed following irrigation, there was observed a rapid increase in oxygen content of the soil atmosphere. The oxygen content of soil air at the 30-inch depth also markedly reflected the irrigation cycles, whereas that at the 96-inch depth was little affected, oxygen content persisting continuously at 15–17 per cent. However, the carbon dioxide content of the soil air at the greater depths maintained itself at about 5 per cent. These data illustrate very well the interrelationship between soil moisture content and composition of the soil air. They are especially striking since they were obtained on a relatively coarse-textured soil—Indio very fine sandy loam. Boynton (*13*) found that fluctuations of ground water in relatively heavy soils was usually fairly well correlated with fluctuations in oxygen and carbon dioxide percentages of the soil air, particularly in the strata of soil just above the water table. Interestingly enough, sometimes a month or more elapsed between the disappearance of ground water and the rise of oxygen level to a maximum. Boynton and Reuther (*14*) recorded oxygen percentages in the soil air as low as 0.1 in the second foot of a Dunkirk silty clay loam under an apple tree during March and April. On subsidence of the water table during the summer, the oxygen content at this depth rose to 17–19 per cent, whereas the carbon dioxide content remained at about 3–4 per cent throughout the year. In the fourth foot, the carbon dioxide content of the soil air remained 8–10 per cent throughout the summer. Excess soil moisture conditions soil aeration which, in turn, affects mineral nutrition.

The essentiality of adequate aeration for the development of healthy roots and vigorous plants of most species has been emphasized many times both in soils (*110, 7, 49, 86, 39, 26, 5, 76*) and in solution cultures (*15, 48, 109, 41*). Numerous studies by Steward and co-workers (*113, 114*), Hoagland and Broyer (*60*), Lundegårdh (*78*), and Robertson and Wilkins (*105*) have amply shown that the rate of nutrient entry into absorbing tissue is conditioned by the rate of respiration of the absorbing cells, which, in turn, is conditioned by the supply of oxygen. Lawton (72) grew corn plants on cultures of Clarion loam and Clyde silt loam in which the moisture regime was maintained at near saturation in some of the cultures and at or below field capacity in others. The inferior corn plants produced by the soils maintained at near saturation were found to have a relatively low percentage composition of nitrogen, phosphorus, and potassium, but there was little consistent effect on content of calcium and magnesium.

Modified soil atmospheres resulting from excess soil moisture affect the nutrition of plants not only through reduced oxygen supply but also by increased partial pressure of carbon dioxide. Bradfield (*18*) has discussed the influence of increasing partial pressure of carbon dioxide within a system containing calcium carbonate. In general, there is an associated increase in activity of both hydrogen and calcium ions. It is conceivable that these effects could markedly affect availability of calcium and also other nutrient ions under certain soil conditions. In fact, McGeorge and Breazeale (*81, 21*) conclude that a supply of carbon dioxide in the soil is the primary consideration in maintaining phosphate availability in calcareous soils as a result of its modulating effect on the high pH of these soils. On the other hand, Parker (*93*) found that on a noncalcareous soil, Norfolk sandy loam, there was no consistent effect on the calcium and phosphorus content of plants whether the soil was fortified with carbon dioxide, the carbon dioxide removed, or the soil left untreated. Chang and Loomis (*28*) have presented evidence that increasing carbon dioxide content of the air-supplied nutrient cultures may be toxic per se to plants over and above any effect of inadequacy in oxygen supply. Increasing partial carbon dioxide pressure would effect an increase in bicarbonate ion concentration in the aqueous phase. Harley and Lindner (*56*) noted that when certain apple and pear orchards in Wenatchee, Washington, were irrigated for a number of years with water high in bicarbonate (200–360 p.p.m.), they showed a marked decline in vigor and an increase in incidence of chlorosis. A marked improvement was noted when irrigation water low in bicarbonate was substituted for the high bicarbonate water. Calcium carbonate concretions developed on the roots of the affected trees when high bicarbonate water was applied to the calcareous soil present in that area. It is of interest to note that the chlorosis which develops under these conditions is characterized by an abnormally high potassium and low calcium content of the leaves (*74*).

The evidence appears to indicate that relatively low concentrations of carbon dioxide in the soil air of calcareous soils aid nutrient availability, but that the high carbon dioxide partial pressures which may occur under excessive soil moisture may be deleterious to root activity and nutrient entry.

Excessive soil moisture may have an indirect effect on the supply of nutrients to the plant. Oskamp and Batjer (*92*) observed in their studies of soil conditions in relation to fruit growing in New York that the most unfavorable orchard locations are those in which shallow rooting occurs because of a high water table during certain seasons of the year. Muller (*86*) also noted that claypan soils maintaining a high moisture content of the subsoil restrict root development of guayule and may even asphyxiate those roots which have penetrated the lower strata prior to the prevalence of excess moisture. That is, shallow and restricted root development means that a given plant has a correspondingly smaller volume of soil to draw upon for nutrients. Furthermore, the moisture reservoir available to the plant is also restricted so that the plant may suffer from drought and be unable to utilize nutrients in the fertile top soil, even though moisture is present in soil at depths within the normal scope of root penetration of the species. Thus, paradoxically, the prevalence of excess moisture in the soil during the early part of a growing season may seriously intensify the adverse effect of drought later in the season.

Under the anaerobic conditions which may prevail in wet, poorly drained soils, there tends to be a decrease in the degree of oxidation of both inorganic and organic constituents. Lawton (*72*) observed a marked increase in the extractable ferrous iron content of Clarion loam and Clyde silt loam when the soils were compacted or maintained at high moisture content together with an associated decrease in extractable ferric iron. Anaerobic conditions resulting from waterlogging of soil may also effect an increase (tremendous in some soils) in exchangeable divalent manganese (*112, 73*). Studies by Fujimoto and Sherman (*43*) indicate that the effect of a level of soil moisture on manganese is complicated by a hydration-dehydration equilibrium. The complex hydrated oxide found in cool, moist soil has a much lower activity than dehydrated divalent manganese. The evidence available is ample to lend weight to the suggestion of Hoffer (*61*) that a relatively high accumulation of reduced iron and manganese in the soil under anaerobic conditions may well be toxic per se to plant roots over and above any effect due strictly to inadequate aeration of the root surfaces.

There is some evidence to indicate that the reduced forms of certain organic components prevailing under the anaerobiosis of waterlogged soils are specifically toxic to plants (*106, 19, 94*), and that these substances are readily oxidized and rendered harmless under aerobic soil conditions. The concept that specific toxicity of certain organic substances in soil has an adverse effect in plant nutrition has been scoffed at many times, but it has been adequately demonstrated by recent investigations (*10, 8, 50*). Thom and Smith (*115*) point out that the anaerobic decomposition of organic matter in waterlogged soils frequently produces hydrogen sulfide. This compound is very toxic to roots.

The presence of accumulations of carbon dioxide in the atmosphere of waterlogged soils exerts a modulating influence over the activities of Fe^{+++} and Fe^{++}, and the consequent influence upon plant nutrition, over and above the effect of the state of oxidation–reduction in the system. Halvorson (55) points out that ferrous carbonate is very insoluble as compared with ferrous bicarbonate which is readily soluble. Thus, the activity of Fe^{++} in a given soil system is conditioned by oxidation–reduction potential, pH, and partial pressure of carbon dioxide within the limitations of the amount and kind of ferruginous mineral present. Halvorson's (55) analysis of the obtaining equilibria indicates that anaerobic conditions in an alkaline soil in the presence of a relatively high partial pressure of carbon dioxide may actually bring about a reduction in solubility of iron as compared with the aerobic state. On the other hand, increased partial pressure of carbon dioxide under aerobic soil conditions is actually conducive to solubility of ferric hydrate.

There is considerable evidence to support Kliman's (*69*) conclusion that iron enters plants mostly in the reduced state. As a consequence, the iron supply to plants on wet soils may be either hindered or accentuated depending on the status of other prevailing conditions (pH, carbon dioxide pressure), and the activity of iron in the soil appears to affect the relative rate of entry of other plant nutrients into the roots.

In light of the foregoing, it is of interest to consider the nutritional disturbance known as "lime-induced chlorosis." There is a great deal of evidence (*25, 80, 54, 99, 1, 85, 34*) that this type of chlorosis, which is associated with a disturbance in iron metabolism within the plant, is accentuated by wet weather or heavy irrigation and is ameliorated when the soil dries. Wet, poorly aerated calcareous soils would be conducive to accumulations of the bicarbonate ion, and it should be recalled that Harley and Lindner (*56*) observed the development of chlorosis on apple trees when irrigated with high bicarbonate water. However, Reuther and Crawford (*99*) found no relationship between the carbon dioxide content of the soil atmosphere and the degree of chlorosis of grapefruit when the intensity of the symptoms varied with soil moisture content over an irrigation cycle. Obviously, the primary cause of chlorosis of plants growing on wet calcareous soils has not been resolved, but a consideration of Halvorson's (*55*) theoretical treatment along with manganese chemistry and HCO_3^- activity might prove fruitful. The status of both iron and manganese in the soil is intimately related to the prevailing biological activity (*55, 73*). Hence, the effect of a high level of soil moisture upon the prevailing microorganisms must be taken into account. As a case in point, Jones and Tio (*67*) observed that symptoms of frenching on tobacco associated with low iron content of the plant could be eliminated by: (*a*) adding ferrous sulfate to the soil, (*b*) maintaining a relatively low soil temperature, or (*c*) by autoclaving the soil. The interrelationship between iron availability to the plant and activity of microflora in the soil is implicit in their findings.

It is apparent, a priori, that wet, poorly drained soils are favorable to the development of anaerobes and inhibitive to aerobes. Since the anaerobes are capable of using oxygen that is in chemical combination with soil components to meet the needs of their life processes, their activity effects a reduction in iron, manganese, and other reducible compounds (*73*). It is also known that denitrification takes place rapidly in waterlogged soils (*131, 37, 134*). There is a rapid loss of applied nitrate under these conditions, but only a fraction of it is recoverable as ammonia. Willis and Sturgis (*134*) observed that large quantities of nitrogen as ammonia are lost from waterlogged soil high in nitrogen and maintained at a high temperature (100° F.), or from soils high in organic matter. These results indicated that such a soil will tend to reach an equilibrium at which it will maintain a low soluble-nitrogen content against losses induced by high temperatures and alkaline reactions. De and Sarkar (*37*) found that much of the difference in nitrogen between the amount of nitrate applied to a waterlogged soil and that recoverable as ammonia was due to nitrogen assimilated by the increased population of microorganisms. Wallihan (*131*) confirmed this and pointed out that this condition explains the relatively low loss of nitrogen when waterlogged soils are drained. He found that there was a relatively rapid rate of nitrate production following drainage of such a soil, providing further evidence that the denitrification process actually prevents excessive losses of an important plant nutrient from waterlogged soils. On the other hand, the nitrogen so stored may be withheld from crop plants, rice for example, growing on such a soil. In fact, Willis and Sturgis (*134*) have emphasized the generally poor response observed to applications of nitrogen on rice. They attribute much of this effect to loss of ammonia on denitrification, but the competition for nitrogen by microorganisms is also undoubtedly involved.

A further effect of wet soils on microorganisms arises from the fact that wet soils tend to be cold (*6*). That is, the higher the moisture content of a soil the higher its heat capacity. This means that wet soils warm up more slowly during the spring months. Since relatively low soil temperatures depress microbiological activity, nutrient availability dependent on this activity will be correspondingly depressed.

MINERAL NUTRITION UNDER SOIL MOISTURE VARIATIONS BETWEEN FIELD CAPACITY AND THE WILTING PERCENTAGE

The mineral nutrition of plants within the available range (see p. 412) of soil moisture is conditioned by (*a*) the extent to which growth and, consequently, mineral utilization might be limited by water supply, (*b*) the effect of change in thickness of the moisture films on nutrient availability, and (*c*) the effect of variations in soil moisture tension upon microbiological activity.

The availability of soil moisture within the available range

It is at once obvious that regardless of how nearly optimal the level of mineral nutrients and other growth factors may be, growth will be limited by the extent to which water supply to the plant is limited. Hence, the question arises: is water ever limiting to growth within the bounds of the available range of soil moisture?

Veihmeyer and Hendrickson (*122, 121*) have carried out extensive investigations on the availability of soil moisture to tree fruits growing on deep alluvial soils in California. Their observations indicated that for all practical purposes, soil moisture between field capacity and nearly down to the wilting percentage is essentially of equal availability to the plant. It is evident, a priori, that more osmotic work is required for the entry of water into a plant when the water is restrained by a force of 15 atmospheres (approximate retentive force at wilting percentage) as compared to a retentive force of only 0.1 atmosphere; i.e., ease of entry of water into a plant may change markedly over the available range. The apparent contradiction between the two previous statements is partially ameliorated by the fact that the relationship between moisture content and moisture retention is invariably hyperbolic (Figure 1); that is, most of the available water is removed from the soil before a marked increase in soil moisture tension develops. Furthermore, whether or not modifications in growth response will be observed with variations in depletion of soil moisture within the available range will depend on (*a*) the nature of the soil, (*b*) prevailing weather conditions, (*c*) kind of plant being studied, and (*d*) the criterion of growth being used.

Deep alluvial soils that permit deep root penetration must be contrasted with shallow soils or dense, impervious soils. Thus, Boynton (*12*) found a definite decrease in growth of apples on a shallow soil in New York, if the soil moisture content of the surface two feet decreased to the wilting percentage. That is, the moisture reservoir for these shallow-rooted apple trees was virtually exhausted under the stated condition. Roots of trees on alluvial soils in California may penetrate 20 feet in depth, so that depletion of the soil moisture in the surface two feet of soil, under such conditions, would not indicate exhaustion of the total moisture reservoir to the tree. Veihmeyer and Hendrickson (*124*) point out that on compact or dense soils, plants may show symptoms of moisture stress even though "available" water is still present in the soil. They attribute this to the poor permeation of roots in such soils. Aldrich *et al.* (*3*) also noted that irrigated Anjou pears on a Meyer clay adobe showed a decrease in growth before the moisture was depleted to the wilting percentage. They regarded the poor root permeation observed in this soil as a partial contributor to this result. It may be shown by a study of moisture sorption curves for different soils that when 75 per cent of the available water is removed, the remaining water may be under a tension of only 1 atmosphere in some soils, but 5 atmospheres in others. Consider the five soils represented in Figure 1. The data are replotted in Figure 4 in such manner that the moisture content at the 15-atmosphere value is designated as "O" available water; the moisture content of the soils at 0.15-atmosphere tension (approximately field capacity) is designated as 100 per cent available water. In other words, the relative scale is simply the moisture present in excess of the 15-atmosphere percentage divided by the total available range as just defined. When moisture retention curves are plotted on this relative basis, it is quite evident that their respective loci vary considerably, depending on the specific nature of the soil. For example, it is shown in Figure 4 that when the moisture tension reached 1 atmosphere in the Indio sandy loam, about 84 per cent of the "available moisture" had been removed; whereas, at this same tension, only about 50 per cent of the "available water" was removed from the Olympic clay. It is reasonable to assume that variations in plant growth associated with variations in soil moisture content above the wilting percentage are more likely to prevail on the latter type of soils than on the former. And there is considerable evidence (*128, 129, 36, 44, 64*) that growth of plants may decrease with a concomitant decrease in soil moisture content of the root zone at moisture levels above the wilting percentage.

The status of prevailing weather conditions may be a determinant as to whether or not decreasing soil moisture content within the "available range" will affect plant growth (*118, 30*). If the level of soil moisture approaches the wilting percentage while the plant is subjected to a cool humid environment, an associated decrease in growth is much less likely than if the plant is growing in a hot, dry environment. That is, at the lower levels of "available moisture" the supply of soil moisture in the former case could probably maintain turgescence, whereas it would probably be insufficient in the latter instance.

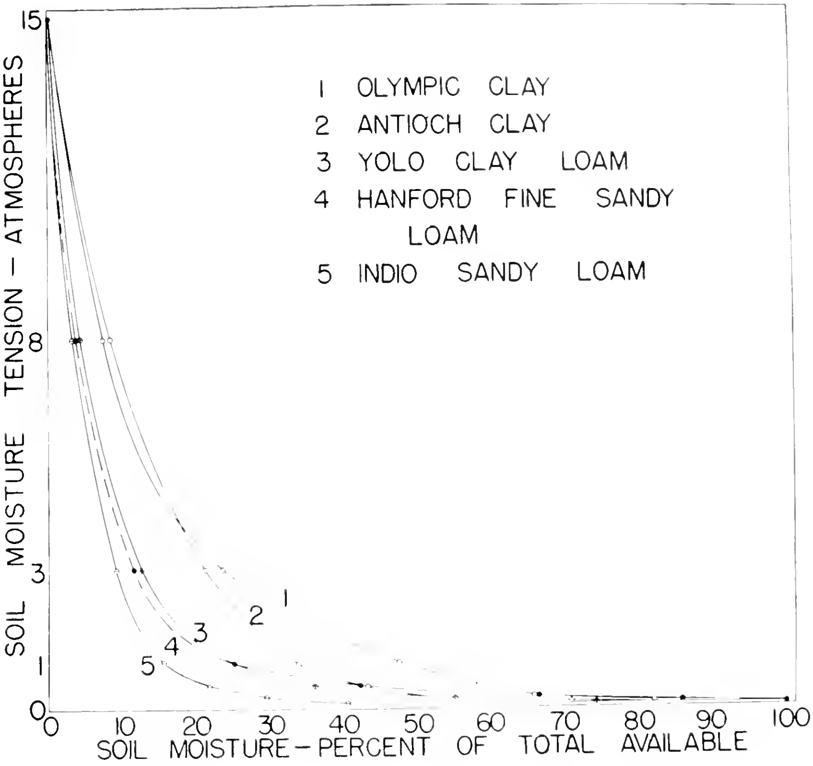

FIGURE 4. Relative loci of moisture retention curves presented in Figure 1.

The evidence indicates that some species of plants, e.g., the tree fruits, show no response in productiveness regardless of the level of soil moisture maintained above the wilting percentage. There are productive vineyards in the San Joaquin Valley of California that receive neither rain nor irrigation during the entire growing season. On the other hand, potato fields in the same area are irrigated daily, and successful maintenance of Ladino clover pastures in this valley requires that the soil moisture content be kept at near-field capacity. The lower soil temperatures prevailing in highly moist soils may be a factor in the need for much more frequent irrigation of potatoes and Ladino clover.

The criterion adopted as the measure of growth response is involved in the evaluation of the effect of degree of soil moisture depletion on growth. Adams, Veihmeyer, and Brown (*2*) studied the effect of various irrigation regimes on growth and productiveness of cotton. The plots maintained at the highest level of soil moisture produced the maximum vegetative growth of the plants, and vegetative growth declined with decreasing soil moisture reserve at time of irrigation. Yet, there were virtually no differences in yield of seed cotton per acre among these various treatments. Thus, the plants made a physiological response to increased level of soil moisture supply, but did not provide a corresponding economic return. Hendrickson and Veihmeyer (*57*) observed the maximum vegetative growth of peach trees on plots that had the most abundant water supply. However, the production of these trees was not superior to those irrigated less frequently, and the keeping quality of the fruit from the frequently irrigated trees was quite inferior. Here again, maintenance of relatively moist soil produced the maximum physiological response as regards vegetation, but the treatment was actually an economic liability. Guayule has been observed to decline in vegetative growth as soil moisture depletion prior to irrigation is intensified, but rubber production was found to be increased (*129, 64*).

The tenability of the concept that all soil moisture above the wilting percentage is "equally available" to plants is conditioned, therefore, by the criteria used in evaluating the results, in addition to the degree of prevalence of modulating factors. Consequently, the question as to whether or not the soil moisture content above the wilting percentage will become limiting to full utilization of nutrients available to the plant is correspondingly involved. Unquestionably, conditions frequently prevail in which plant response to fertilization is limited by soil moisture supply within the available range, but any generalization would be hazardous, considering the present state of our knowledge of the subject.

Nutrient accumulation in plants at various levels of soil moisture supply

It would be logical to conclude that under conditions of adequate nutrient supply, plants that are limited in growth by a relatively low level of soil moisture would have a higher content of mineral nutrients than plants under comparable fertility but not limited in growth by moisture supply. Miller and Duley (*84*) studied this relationship on corn plants. The growth of corn at an "optimum" level of soil moisture was greater than that of comparable plants in soil maintained at a "minimum" level of soil moisture. The nitrogen, phosphorus, and potassium content of the plants was appreciably higher under the "minimum" soil moisture level, but the reverse relationship was observed for calcium. Emmert (*40*) found that the smaller tomato plants grown with a relatively low soil moisture supply were higher in nitrogen and potassium content and lower in phosphorus content than the larger control plants grown at an optimum level of soil moisture.

Many studies have provided evidence somewhat at variance with that cited above. Haddock* found no consistent variation in nitrate content in petioles of sugar beets subjected to wide differences in irrigation regime. Leamer *et al.*† found that alfalfa irrigated whenever the soil moisture tension reached 0.4 atmosphere at the 1-foot depth had a slightly higher nitrogen content than comparable alfalfa on which irrigation was delayed until moisture tension reached 4.0 atmospheres at the 1-foot depth. The effect of soil moisture content on activity of nodule bacteria may be involved here. Most experimental evidence shows that for a given level of fertility, decreasing soil moisture supply is associated with a definite increase in nitrogen content of the plant tissue, a definite decrease in potassium content, and a variable effect upon content of phosphorus, calcium, and magnesium (*117, 116, 82, 66,*

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*J. L. Haddock. Personal communication of an unpublished report.

†R. W. Leamer, S. R. Olsen, C. R. Domingo, and C. A. Larson. Personal communication of an unpublished report.

52, 53). In other words, it is well established that when growth of plants is limited by soil moisture supply, nitrogen tends to accumulate within the plant because the rate of entry is approximately maintained in conjunction with a decreased rate of utilization in growth processes; but the general tendency for potassium content to be relatively low in plants on the drier soils shows that rate of entry of potassium decreases to a greater degree than does rate of utilization in these slower growing plants. Hence, the availability of potassium to plants may be depressed at the lower soil moisture contents, depending on the nature of the soil. It is of interest to note, however, that Wimmer *et al.* (*136*) studied nutrient content of two varieties of sugar beets at different soil moisture levels and reported that one variety showed the conventional decrease in potassium content with decreasing moisture supply, whereas the other variety showed the reverse trend under the same conditions. If this observation is verified, it will indeed be a remarkable case of specificity in ionic entry between two varieties of a given crop.

Although the phosphate ion may accumulate in plants limited in growth by low soil moisture supply (*84, 66*) there is also evidence* that plants so affected may have a relatively low content of phosphate (*116, 117, 40*). Thus, the effect of soil moisture on phosphate nutrition is far less consistent than that observed for nitrogen or potassium. This seems to be indicative of the wide variation among soils in their fixing power for phosphorus as conditioned by soil moisture content. Miller and Duley (*84*) grew corn on a fertile silt loam from an alluvial bottom along the Missouri River and, Janes' (*66*) bean plants were grown on an Arredondo loamy sand fertilized with 1200 lb. per acre of 4-7-5. These soils were conducive to phosphate accumulation under low moisture supply.

McMurtrey *et al.* (*82*) grew tobacco on a Collington fine sandy loam fertilized with 750 lb. per acre of 4-8-12 in the row, and found no effect of soil moisture supply on phosphate content of tobacco leaves. Haddock* grew beets on a calcareous soil, Millville silt loam; Thomas *et al.* (*116*) studied tomatoes presumably grown on a Hagerstown silty clay loam that was variously fertilized. These two experiments yielded evidence that the phosphate content of plants was reduced by diminishing

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*J. L. Haddock. Personal communication of an unpublished report.

the soil moisture supply. It is quite probable that these various soils differ considerably in their fixing power for phosphate and that this variation is related to the observed effects of soil moisture supply on phosphate content of plants.

The available evidence consistently shows magnesium to be relatively high in plants growing under restricted soil moisture supply (*116, 82, 66*). This is in line with the inverse tendency for magnesium deficiency to develop in plants during periods of heavy rainfall.

Since there is a tendency for the entry of calcium and potassium into plants to vary reciprocally, it could be inferred that the characteristically low potassium content of plants with inadequate soil moisture supply would be accompanied by a relatively high content of calcium. McMurtrey *et al.* (*82*) and Thomas *et al.* (*116*) found this to be the case on fertilized soils, but the latter investigators found the reverse trend on their unfertilized plots. Miller and Duley (*84*) and Janes (*66*) found virtually no effect of soil moisture supply on calcium content of their experimental plants. It is evident, therefore, that the status of other constituents in the soil have a modulating effect on calcium availability under varying soil moisture content.

As pointed out in the first part of this paper, diminishing soil moisture content effects a concentration of the solutes in the soil solution. Fertilized plants may even intensify this solute concentration as a result of increased rate of moisture extraction. Thus, Jordon *et al.** found that corn plants fertilized with nitrogen during a dry year not only rapidly depleted the soil moisture to the wilting percentage in the surface foot of soil, but also to the 3-foot depth. On the other hand, readily available moisture continuously prevailed in the 3 feet of soil under the unfertilized control plants. In a well-fertilized soil subjected to a prolonged dry spell, this solute concentration may in itself inhibit water availability, so that growth on the unfertilized soil may be better than that on which fertilizer was applied. Neff and Potter (*87*) noted that newly transplanted tung trees were injured by mineral fertilization during a dry year. Carolus and Woltz (*27*) found that during four dry seasons in eastern Virginia, the more nitrogen fertilizer they added to

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*H. V. Jordan, K. D. Laird, and D. D. Ferguson. Personal communication of an unpublished report.

a Sassafras sandy loam, the lower the yield of potatoes. Adding increasing amounts of superphosphate had a moderately beneficial effect, however. The effect of these two fertilizing materials on potato yields under these conditions was directly related to their effect on the solute content of the soil solution. Correspondingly, Rahn (*96*) found that during a "dry" year, fertilization with manure produced a much higher yield of melons than did mineral fertilization, but that during a "wet" year there was no difference in effect on yield from these two sources of fertility.

The need for taking into account soil moisture supply in adjusting the fertility program is well recognized in the Hawaiian Islands. Nightingale (*90, 89*) emphasized in his studies on the nitrogen, phosphorus, and potassium nutrition of pineapples that the capacity of the plant to utilize efficiently the available supplies of these nutrients was conditioned by soil moisture supply and other environmental factors. Clements and Kubota (*31, 32*) have developed a technic for following the status of moisture, nutrient, and sugar content of sugar cane over the course of its development, and of adjusting the irrigation and fertility program in accordance with the trend in the status of the plants. Dr. Clements discusses this technic in an accompanying paper.

The effect of change in thickness of the moisture films on nutrient availability

The discussion in the fore part of this paper pointed out that as the thickness of moisture films on the soil particles decreases, the intensity with which the water is retained on the particles by surface force action increases. Buehrer and Rose (*24*) discuss the physical properties of adsorbed water. The initial layer of water adsorbed on clay particles is presumably held by a "pressure" of several thousand atmospheres (pF 6–7). The characteristics of water retained under such high pressure differ from those of "free" water. There is found to be a tremendous drop in the dielectric constant and presumably a decrease in its solvent power because of the greatly decreased polarity. This implies, therefore, that with diminishing thickness of moisture films, there is a corresponding decrease in the proportion of water in the film with normal solvent properties, i.e., "unbound" water. Reitemeier (*98*) determined the dissolved ions in solutions extracted from six soils at four moisture contents, and found that the concentration of nitrate and chloride increased as the moisture content decreased. He explained this effect on the basis of the existence of "unfree" water in the soil or negative adsorption of monovalent anions, or a combination of both. This relative concentration of chloride and nitrate ions in the outer layers of thin moisture films may also be involved in the usually observed accumulation of nitrogen in plants on dry soils. In general, Reitemeier found the opposite effect for cations and polyvalent anions; that is, as the thickness of the moisture films decreased, there was a relative diminution of the concentration of these ions in the outer layers. It may be coincidental, but plants subjected to low soil moisture supply also tend to have a relatively low content of these ions (*52, 53*).

The diminished amplitude of the cationic swarm about an adsorption surface under a thinning moisture film appears to be conducive for potassium ions present to enter the lattice of the clay crystal and become fixed. Volk (*127*) found that alternate wetting and drying of soils treated with soluble potassium salts caused rapid fixation of potassium in a nonreplaceable form, and that very little fixation of this kind took place when the soils were kept continuously moist. This has been verified many times (*97, 133, 4, 111*). Acid soils fix relatively little potassium when moist, but drying effectively increases potassium fixation. Calcareous soils fix potassium when moist and the extent of fixation increases on drying. Martin *et al.* (*83*) suggest that the effect of drying on potassium fixation is that of increased concentration of ions at the adsorption interface, and that dehydration, per se, is not involved. It is logical to conclude that the relatively low potassium content of plants subjected to low soil moisture supply is related to the increased intensity of potassium fixation under such conditions.

Soil moisture depletion is also conducive to the fixation of phosphorus (*88, 119*). Trumble (*119*) concludes that this explains the relatively low phosphorus content of plants under inadequate soil moisture supply. Neller and Comar (*88*) report that the extent of phosphorus fixation on drying is directly related to the clay content of soils.

Effect of variations in soil moisture tension upon microbiological activity

The important role of microorganisms in the mineral nutrition of plants is discussed in an accompanying paper by Dr. Norman. It follows, therefore, that any effect that varying degrees of soil moisture tension would have on microbial activity may result in an indirect effect on mineral nutrition. There have been numerous studies (*11, 29, 51, 132*) on the relation of soil moisture to soil microorganisms, but as pointed out by Bhaumik and Clark (*9*), in most of this work the soil moisture levels were expressed as a percentage of the maximum water-holding capacity. This technic often fails to ensure even moisture distribution throughout the soil sample, or to maintain constant moisture content over the experimental period.

Bhaumik and Clark (*9*) adjusted the moisture tension of samples of five different soils at 0, 0.001, 0.01, 0.05, 0.5, and 3.2 atmospheres, and collected the carbon dioxide evolved during the course of incubation. In two soils the peak rate of carbon dioxide production was at 0.5 atmosphere of moisture tension, and in the other three soils at 0.05 atmosphere of tension. For all soils, the peak rate of carbon dioxide production was observed at or very near to the moisture tension at the aeration porosity limit, taken by convention as 0.05 atmosphere. Total carbon dioxide production was actually at a maximum in Thurman sand at the highest moisture tension used, and it was relatively very low at the lowest two levels of moisture tension. On the other hand, total carbon dioxide production for the Wabash silty clay was only slightly less than maximum on the saturated soil, but was relatively low at the highest moisture tension. The diverse effect of moisture tension on microbiological activity on these two soil types differing widely in texture is indeed intriguing. This difference may be partially explained by the enormous increase in population of fungi at 3.2 atmospheres tension, as compared with zero tension in the sample of Thurman sand. Novogrudsky (*91*) studied the rate of nitrification in a chestnut soil as a function of moisture content. No nitrification occurred when only hygroscopic water was present, but it was evident when the moisture content was equal to about 1½ times maximum hygroscopicity (presumably slightly above the wilting percentage). Nitrification reached its greatest intensity when the upper limit of film water equaled twice the maximum molecular water-holding capacity (presumably twice field capacity); these results tend to be in line with those of Bhaumik and Clark (*9*) for their heavier soils.

Waksman (*132*) has reviewed the earlier work on the influence of soil moisture on microbiological activity. Different organisms vary as to the optimum soil moisture content for their activity. Thus, nitrification is at its highest near moisture content of field capacity, and excessive quantities of water are much more injurious than too low a moisture content. It is quite evident that to whatever extent the mineral nutrition of plants is dependent upon the activity of soil microorganisms, soil moisture level will have an indirect effect on nutrition through its influence on soil microbes.

Another aspect of the relation of soil moisture to microorganism activity is concerned with minor element nutrition. The mineral nutrition of crops on sandy soils in Wisconsin is intimately connected with the organic matter content of these soils. It has been reported to the authors that during the drought year of 1946, boron deficiency symptoms became prevalent throughout the state. It could have been that reduced microbial activity due to drought caused insufficient mineralization of the boron present in the organic matter of the soils.

MINERAL NUTRITION IN DRY SOILS

During protracted periods of drought, the fertile surface soil may dry to less than the wilting percentage. This may have drastic consequences to shallow-rooted crops. Even though deep-rooted plants may obtain adequate moisture under these conditions from the deeper horizons, the question immediately arises as to the ability of plants to absorb nutrients from the fertile top soil when it is drier than the wilting percentage. For example, boron deficiency symptoms usually become predominant during a drought (*95, 22*). There are two alternative explanations for this: (*a*) Drying of soil may affect the availability of borate, as is the case with potassium and phosphate; or (*b*) plants are unable to absorb boron when the soil moisture is below the wilting percentage and inadequate supplies of this nutrient are present in the lower horizons.

Breazeale (*20*) carried out a number of experiments to ascertain whether or not plants are able to remove nutrients from soil that is drier than the wilting percentage. When the root system of a plant was divided between a moist soil and soil with moisture content below the wilting percentage, Breazeale (*20*) noted the root system transported water from the moist to the dry soil, raising the moisture content of the latter to slightly above the wilting percentage. He also found evidence that because of the moisture transfer phenomenon, the roots were able to absorb potassium from the dry soil. That is, net movement of potassium into the roots was in the direction opposite to the net movement of water out of the roots and into the dry soil.

A pertinent consideration in the ability of roots to remove nutrients from dry soils is whether or not the roots are able to grow and proliferate in the dry soil. Loomis and Ewan (77) found that the roots of 29 different genera of seedlings completely failed to penetrate soil at a moisture content of about the hygroscopic coefficient. Hendrickson and Veihmeyer (58) concluded that the roots of sunflower plants would not grow into soil drier than the wilting percentage. Shantz (*107*) also concluded that the roots of crop plants lack the ability to penetrate dry soils, but he believed that the roots of xerophytic plants possessed this capacity. Lobanov (75) found appreciable variation in the dryness of soil limiting to the growth of roots of woody plants. The minimal soil moisture contents (soil moisture retentive properties unknown) for root growth were 6.11 per cent for *Fraxinus excelsior*, 5.11 per cent for *Caragana arborescens*, and 2.07 per cent for *Picea abies* and *Pinus sylvestris*.

Hunter and Kelley (*62*) have devised an improved technic to study the entry of roots into dry soils and removal of nutrients therefrom. They grew corn plants in tar-paraffin pots filled with moist soil and surrounded with air-dry soil containing radiophosphorus (*63*). In all cases the corn roots penetrated the walls of the pot and extended into the air-dry soil. The moisture content of the "dry" soil increased, but values as high as the wilting percentage were not obtained. The results of this experiment indicated that the roots of corn are able to elongate into dry soil and to build up the moisture content of that soil, but the evidence obtained did not indicate the absorption of nutrients from dry soil by plants. In another experiment, Hunter and Kelley (*62*) obtained an indication that there was a movement of radiophosphorus into alfalfa roots from surface soil at or below the wilting percentage when moisture was available to the roots below the 2-foot depth.

The foregoing observations agree with those obtained by Volk (*125*) using a comparable technic. He also found that corn roots would penetrate a soil when at lower moisture content than the wilting percentage; and, what is especially noteworthy, Volk (*125*) demonstrated that these corn roots were able to absorb appreciable quantities of nitrogen from the dry soil. Furthermore, he found good evidence that significant quantities of potassium and phosphorus may be absorbed from a soil at the wilting percentage, even though the amounts were relatively small as compared with nutrient entry from moist soil.

Although there is some evidence that plants may absorb small quantities of nutrients from dry soils, this source appears to be inadequate for a thriving plant. If moisture is available in unfertile lower horizons of a soil and if most of the fertility is in the surface horizon that has become very dry, the plant may suffer from inadequate mineral nutrients.

SUMMARY

Consideration is given to the physical condition of moisture throughout the plant-growth moisture range, including typical curves showing soil moisture content at moisture tensions up to 20 atmospheres. Principles governing the flow and distribution of water in soils are discussed, together with the relation of field-capacity to the unsaturated permeability of soils.

The mineral nutrition of plants is reviewed under three soil moisture categories: (*a*) excess moisture above field capacity, (*b*) moisture variations within the field moisture range, and (*c*) at moisture content below the wilting percentage.

Excess moisture affects mineral nutrition by loss of nutrients through surface runoff, excessive leaching, and by decreased availability of nutrients under the poor aeration prevailing in wet soils.

Within the field moisture range, soil moisture supply may itself become the limiting factor to plant growth so that nutrients available are not efficiently utilized. Drying of soils intensifies potassium and phosphorus fixation, and lowers their availability. Since soil moisture vari-

ation affects microbial activity, nutrient availability arising therefrom is correspondingly affected.

There are indications that roots may penetrate soils when drier than the wilting percentage and absorb nutrients therefrom, but the amount available under such conditions appears to be of minor importance.

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CHAPTER

18

Environmental Influences on the Growth of Sugar Cane

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Good yields of sugar from cane grown in Hawaii range from as little as 6 tons of sugar per acre to more than 18 tons per acre per two-year cycle. Sugar cane is grown at elevations ranging from sea level on up to 2000 feet. It is produced in areas where rainfall varies from a few inches to 200 inches per year. About half of it is irrigated. It is grown on practically level land and on steep slopes facing all directions of the compass, in hot areas and in cool areas, in bright sunny areas and in cloudy areas. It is produced on soils ranging from residual clay soils to alluvial soils made up of coral, coral sand, and clay.

It is not surprising, therefore, that yields should vary a great deal. It is another matter to determine the factors which cause the extreme variation and to measure the relative influence which each of the several factors has on the production of sugar. Early in my studies at the University of Hawaii (*1*), I grew cane in two different areas—one on cloudy windward Oahu, the other on the sunny leeward. The yields were strikingly different. On the cloudy side about half as much cane was produced as on the other side. In order to evaluate the soil influence, soil was taken from the cloudy area to the sunny area and placed in large concrete pots alongside of other pots filled with soil from the sunny area. Cane was planted and the necessary fertilizers applied equally to the pots. After a while it became apparent that the cane grew equally well in both soils. In fact, what small differences existed favored the soil taken from the cloudy area where normally it produced about half as much plant growth as the other soil under its sunny conditions.

Soils by themselves appear to be a very minor cause of the variation in yields obtained. In order to account for the variations it is necessary to look to the total environment of the plant, both external and internal (*6*). Such an external environment will include temperature, radiant energy, moisture, wind, humidity, and soil. The internal environment is the physiological status of the plant as related to its age, vigor, and composition, with particular reference to moisture, the critical elements, carbohydrates, and, of course, the plant's heredity. The problem from a practical viewpoint then is to attempt a measurement of all the factors influencing growth of sugar cane and to devise a method which will enable the grower to culture his crop taking full advantage of all favorable factors and lessening or overcoming all the unfavorable ones.

At the outset, it was assumed that to obtain a measure of the well-being of a crop, it was necessary to find this reflected in the crop itself. Criteria based solely on soil chemistry except in a few cases have been of little value to an understanding of the physiological requirements of a crop. Just so, it appeared unlikely that the mineral nutrient levels of leaves would solve the problem.

SELECTION OF TISSUES FOR TESTS

The first important task was a thorough study of the several organs or tissues of the plant in relation to the various materials and processes pertinent to growth in order to select that tissue which is the most reliable index to use in following the welfare of the crop being grown. The plant was separated into its several parts: the meristem, the elongating portions of the stem, the mature portion of the stem still bearing living leaves, the old stem from which the leaves had already fallen (divided into three internode units), the unfolding leaves, the young mature leaves divided into blades and sheaths, and the old green leaves also divided into blades and sheaths. Each of these tissues or organs was analyzed for weight, moisture, dry matter, total sugars, hydrolyzable carbohydrates, total nitrogen, potassium, phosphorus, calcium, and magnesium.

Plants grown for these preliminary studies were collected in the early morning at five-week intervals from each of sixteen crops grown at two places, started at four different times of the year. After all the analytical data were assembled—and this involved well over 100,000 items—each tissue was examined statistically to permit the selection of the most reliable tissue for each of the materials considered cogent to the problem. As a result, the leaf sheaths of the young mature leaves (leaves 3, 4, 5, and 6, counting down from the tip leaf as number 1), were found to be the best tissue for tissue moisture (5), total sugars (6), potassium (*4*), phosphorus (*3*), calcium, and magnesium. In addition, the fluctuations in the size of this sample reflected remarkably well the girth of the stalk being laid down at the time the sample was taken. The green tissue of these same leaves was best for use as an index to the nitrogen status of the plant (7).

During the four years in which these collections were made, measurements of labeled pilot plants were made for vertical elongation. The rate of new leaf emergence and the length and width of leaves were determined.

INFLUENCE OF NUTRIENTS, MOISTURE, AND OTHER GROWTH FACTORS

After having determined the most reliable tissue to be used as an indicator of the crop's welfare, the next stage of the work involved the determination of adequate levels of nutrient materials and moisture. These experiments were, for the most part, conducted on the plantation. The results of these studies show that for potassium, 2.25 per cent is the adequate level for the heaviest as well as the lightest production areas (*4*). For phosphorus 0.080 is the adequate level (*4*). Not enough work has been done to fix the levels for calcium and magnesium, but these two materials have not as yet been found to affect cane yields in Hawaii.

The correct levels for nitrogen vary rather markedly from area to area (*3, 6*). In general, the lower the temperature of the area, the higher the nitrogen index of the cane leaves. To be sure, it will take less nitrogen as fertilizer in cool areas to raise the nitrogen index than it will in areas of heavy growth. The relationship between the nitrogen index and the moisture index is so intimate that it is usually easy to determine the correct nitrogen level from actual tissue moisture levels. This applies not only to different climatic zones but also to different varieties within a zone.

In order to determine the relationship of moisture to growth, a new study was undertaken beginning in 1943 and continuing through nine crops (four plant and five ratoons) until 1947. The four plant crops were started at intervals of three months beginning in April, 1943. It is desirable to do this if one is to use the method of multiple regression in evaluating the pertinent factors in the study. If fortuitous relationships are to be avoided, it is desirable in the collection of data to avoid fortuity of circumstances. In this work, eleven samples made up of individual cane tops were collected twice weekly from each of the concurrent crops. Twelve pilot plants were measured for growth elongation in each crop. At the end of each five-week period the total growth of these pilots was adjusted to the total measured from forty other pilots in each crop. Soil borings were made twice weekly in triplicate from each crop, one boring extended down to eighteen inches and a second another eighteen inches downward. Soil moisture determinations were made from these. Toward the end of these studies, tensiometers (*9*) became available and these were included.

Complete weather data were taken from the Hawaiian Sugar Planters Association weather station within a few yards of the plots on which the cane was grown; the data included maximum and minimum temperatures, sunlight measurements as gram calories per square centimeter per day, wind velocities, and humidity records.

Since these crops were being controlled by their own crop logs, regular five-stalk samples were taken at five-week intervals from each crop beginning as soon as the plants were large enough to be sampled and continuing until harvest time. The fertilization of these crops followed crop-log practices (*3*).

After all these data were assembled, they were subjected to statistical analysis using the methods of multiple regression. The first set of partial regressions offered in Table I deals with the several factors as they vary with the growth units. The growth unit is defined as the daily increment increase in the volume of cane produced in a field with a full stand. It is approximated by multiplying the daily elongation rate by the green weight per stalk of the sheath sample. The resulting value, the growth unit, correlates very well with the actual volume of cane laid down. The regression of this value on the actual volume has a "*t*" value of 17.98 and a correlation coefficient of 0.954.

It is apparent that a very high proportion of the variation has been accounted for and that a growth-unit equation predicting yields is possible as we shall see later. The two dominant factors are the green weight of the sample and the rate of leaf emergence. The factor, leaf emergence, is in reality a measure of the plant's vigor and the morphological mechanism of growth. The green weight of the sample again is a measure of the plant's vigor and its girth.

TABLE I

Partial Regressions of Certain Factors on Growth Units

($n = 1373$)

Factor	Beta	"t" Value
Green weight of sheaths	+.3619	17.24**
Rate of leaf emergence	+.2297	10.59**
Maximum temperature (°F.)	+.1724	10.51**
Minimum temperature (°F.)	+.1365	8.58**
Sheath moisture	+.2239	7.98**
Age	−.1166	5.36**
Total sugars	+.0360	2.27*
Soil moisture	+.0276	2.06*
Light	+0.142	.77
Leaf nitrogen	−.0068	.28
	$R = .8999$	

NOTE: Definition of symbols—n, number of observations; Beta, partial regression; *significant (5 in 100); ** highly significant (1 in 100); R, multiple correlation coefficient. These symbols are used in Tables I–VII in this chapter.

The next two dominant factors are the maximum and minimum temperatures. The higher the day and night temperature, the faster the growth rate, other things being equal. This relationship agrees very well with our general experience, although there is a possibility that in a few areas in the islands the maximum temperatures become excessive.

Sheath moisture is the single plant component which achieves high statistical significance as a growth factor. It is dominant over the nutrient material, nitrogen, and also over potassium and phosphorus. Experiments have shown us that where the potassium index is 2.25 or above and the phosphorus index is at 0.080 or above, no increases in growth are obtained by application of these two materials. In these studies, the crops were kept above these limits and, hence, these two materials were not included in the statistical treatment. Throughout my work, the sheath moisture level as a physiological factor dominates not only the growth of the plants but also the levels of the nutrients which the plant maintains. It is so dominant that one is forced to wonder at the completeness of fertilizer trials which ignore this factor. The age of the crop shows a strong negative influence. Perhaps age is not so important as height which, of course, parallels age. The total sugars show a small positive influence on the growth units. Soil moisture achieves minor significance at this point, although we shall see its true significance later. Light intensity is not a significant factor at this point nor is nitrogen.

EVALUATION OF GROWTH FACTORS

Before a complete evaluation of all these factors can be made, it is essential that a measure of influences affecting each of these be looked into. Since growth is determined by morphological structures as well as physiological processes and ecological influences, it is likely that certain factors may not be properly measured when analyzed only against total growth. Thus, for example, radiant energy is shown not to account significantly for any of the variations in growth. Obviously this cannot be true. The same holds true for nitrogen. It is possible that these factors favor one aspect of growth and hamper another, and, thus, in a single analysis would be canceled out, or that the influence of one factor is absorbed by another factor, more dominant in its influence.

In Table II the above factors are measured against the elongation of sugar cane.

The factor most closely related to elongation is the rate of leaf emergence which is, of course, to be expected since leaf and internode formation result in the formation of intercalary meristems and, in turn, compose the morphological mechanism of elongation. The three other dominating factors are sheath moisture, maximum temperatures, and minimum temperatures which are all positive and strong in their influences. The green weight of the sheath tissue, soil moisture, total sugars—all exert significant positive influences while age and nitrogen

TABLE II

Partial Regressions of Certain Factors on the Rate of Elongation

(n = 1373)

Factor	Beta	"t" Value
Rate of leaf emergence	+.4060	17.47**
Maximum temperature (°F.)	+.1976	11.24**
Sheath moisture	+.3283	10.90**
Minimum temperature (°F.)	+.1282	7.51**
Total sugars	+.0934	5.50**
Green weight of sheaths	+.1062	4.72**
Age	−.0981	4.21**
Soil moisture	+.0493	3.42**
Leaf nitrogen	−.0593	2.25*
Light	−.0245	1.25
	R = .8839	

exert negative influences. Radiant energy is not significant at this point.

In Table III a similar analysis of factors is reported dealing with the green weight of the sheaths making up the sample. It should be remembered that this factor, while of no particular moment in itself, is very closely correlated with the thickness of the stem and hence is associated with an element of growth.

TABLE III

Partial Regressions of Certain Factors on the Green Weight of Sheaths

(n = 1373)

Factor	Beta	"t" Value
Sheath moisture	+.7342	16.52**
Age	−.4612	12.50**
Light	+.3616	11.42**
Leaf nitrogen	−.4558	10.61**
Soil moisture	−.1021	4.06**
Maximum temperature (°F.)	−.1184	3.86**
Rate of leaf emergence	+.0808	1.97
Total sugars	−.0501	1.67
Minimum temperature (°F.)	+.0382	1.26
	R = .8268	

The most dominant factor here is sheath moisture. Sunlight asserts itself as another dominant factor. Age is also more important than heretofore. The nitrogen level assumes importance but curiously enough is negative in its influence, as are soil moisture and maximum temperature. The rate of leaf emergence, total sugars, and minimum temperatures are not significantly related to the girth of the stalk.

TABLE IV

Partial Regression of Certain Factors on the Rate of Leaf Emergence
(n = 1373)

Factor	Beta	"t" Value
Minimum temperature (°F.)	+.2716	15.31**
Age	−.3658	14.38**
Maximum temperature (°F.)	+.2507	12.90**
Soil moisture	+.1633	10.20**
Light	+.1503	7.02**
Leaf nitrogen	+.1967	6.66**
Sheath moisture	−.0107	.32

In Table IV is reported the analysis of the factors affecting the rate of leaf emergence. Leaf production provides the plant with its meristems and additionally is a measure of the plant's vigor. As might well be expected, age is a dominant factor and is, of course, negative. Both day and night temperatures are dominant. Soil moisture here assumes a very important role. In fact, it appears that after the plants have used up about two-thirds of the available water there is a striking retardation of leaf emergence and elongation as well. Light intensity and the nitrogen level are also important factors in leaf emergence and vigor. Sheath moisture which has been a dominant factor in all the categories of growth is not significant here.

Thus far we have been determining the weight of factors affecting the growth and vigor of sugar cane. Since the sheath moisture and nitrogen levels are two practical keys to the manipulation of the physiological status of the plant, a measure of factors affecting these levels is worth while. In Table V the analysis of factors affecting the level of sheath moisture is given.

Soil moisture, being the source of plant moisture, is of course primary, but there are other factors which are important in determining the moisture level of the tissues. However, in these studies the soil moisture was maintained well above the wilting point throughout the crop except toward the end when the crop was put on a drying schedule.

Under these circumstances the dominant factor affecting the moisture level is the nitrogen level. The strong positive relation between maximum temperatures of the air and the moisture level may be somewhat surprising but shouldn't be so when it is remembered that low soil temperatures which follow air temperatures may result in actual desiccation of the plants even though soil moisture is adequate. Light is strongly negative in its influence on tissue moisture, as is age. The humidity relationship is strongly positive as it should be, and it appears that this factor has not been given enough attention in the past. In this analysis wind velocities are comparatively minor but are shown to be positive. However, in the Islands high winds are associated with stronger light intensities and lower humidities and, thus, the influence of wind may be masked, especially since the area in which this study was carried on is not a high wind area.

TABLE V

Partial Regressions of Certain Factors on Sheath Moisture

($n = 1373$)

Factor	Beta	"t" Value
Leaf nitrogen	+.6464	30.97**
Maximum temperature	+.2409	12.15**
Age	−.1825	8.81**
Light	−.1721	6.72**
Relative humidity	+.1245	6.46**
Soil moisture	+.0800	5.20**
Wind velocity	+.0727	3.54**
	$R = .846$	

In Table VI a similar analysis of factors affecting the nitrogen level is shown.

The outstanding factor affecting the nitrogen level is tissue moisture. Age is also dominant. In fact, after a crop is in its second year of growth, it is difficult to affect the nitrogen level by application of fertilizer.

Minimum temperatures exert a strongly negative influence while maximum temperatures exert a weaker positive influence. Soil moisture is also negative but its influence is weak. Light does not seem to affect the nitrogen level.

TABLE VI

Partial Regressions of Certain Factors on Leaf Nitrogen
(n = 1373)

Factor	Beta	"t" Value
Sheath moisture	+.6334	29.49**
Age	−.2932	14.28**
Minimum temperature (°F.)	−.1262	7.06**
Maximum temperature (°F.)	+.0858	4.43**
Soil moisture	−.0570	3.55**
Light	+.0126	.65
	R = .832	

It is evident from our analysis so far that the elements of weather have an overwhelming influence on the growth of sugar cane. When the physiological status of a crop can be controlled by maintaining the moisture and carbohydrate levels at known levels for each stage of growth, it is apparent that the yields which are obtained should be maximal for a particular location and should be related, not so much to the so-called fertility of the soil but to the energy level of the atmosphere as composed of radiant and heat energy. To be sure, we can hope to produce the maximum crop only occasionally, since every storm, every unfavorable circumstance of soil, weed control, or general mismanagement of a crop will reduce the likelihood of the maximum achievement by amounts in direct proportion to the sum total of mishaps. We ought, however, to be able to predict what can be achieved in any given area and proceed from this to narrowing the differences between the actual and the theoretical yields.

In order to develop this equation for sugar cane, I have taken the factors of maximum and minimum temperatures, radiant energy, and the two dominant physiological factors, tissue moisture and age, and have calculated the partial regressions for growth units. Vigor of plants is not directly used here, although it will be remembered that among other factors, moisture and age were dominant influences on factors of plant vigor. The results of this analysis are shown in Table VII. The five factors show a well-balanced assumption of influence on growth units.

TABLE VII

Partial Regressions of Certain Factors on Growth Units
($n = 1373$)

Factor	Beta	"*t*" Value
Light	+.1888	10.74**
Sheath moisture	+.4217	21.53**
Age	−.3552	18.97**
Maximum temperature (°F.)	+.2035	11.50**
Min mum temperature (°F.)	+.2320	14.22**
	$R = .8617$	

From these data, the following equation evaluating the weight of each factor on growth units was obtained:

$$E = 0.0820X_1 + 9.3206X_2 - 0.0979X_3 + 2.8025X_4 + 3.2272X_5 - 1130.9004$$

where X_1 is the average daily radiant energy expressed as gram calories per square centimeter per day; X_2 is the sheath moisture expressed as percentage of green weight; X_3 is the age expressed in days; X_4 is the maximum daily temperature as degrees Fahrenheit; and X_5 is the minimum temperature expressed in degrees Fahrenheit. E is the estimated average number of growth units produced per day.

When this equation is applied to the pertinent data collected in this study and the average daily growth units are multiplied by the actual age of the crop in months, the results are as shown in Table VIII.

The fact that the averages of the two columns are so very close is encouraging. Discrepancies between items in the columns means that more work has to be done toward achieving greater accuracy. However, the results are sufficiently good to justify projecting the equation to several of the plantations with which I am associated to determine what yields ought to be obtained at each.

In arriving at these estimates I used the temperature and light measurements as obtained at each place. Age would, of course, be the same for all the estimates. For the moisture values I used first that level which is near the ideal, and second, levels which were actually obtained at each place.

TABLE VIII

Actual *vs.* Calculated Growth Units

Plots	Actual	Calculated
A	1580	1454
B	1951	1882
C	2149	1938
D	2124	2045
RA	1194	1358
RA	1123	1251
RB	1841	1990
RC	1717	1739
RD	1689	1695
Average	1708	1706

The conversion of growth units to tons-cane-per-acre was accomplished by multiplying the average growth unit per day for the crop by the age of the crop, that is, 24.0 months. The resulting value was converted to tons-cane-per-acre by multiplying by the conversion factor 0.065. The results are shown in Table IX. In the first column are given the theoretical yields for the light and temperatures actually experienced at the nine places listed, using the same normal moisture curve for each. In the second column the same local light and temperature records were used but the actual moisture level measured on a good crop at each place was used. In parentheses, after each of these yields is the actual yield obtained on the field from which the moisture levels were taken. The agreement of the two sets of values in the second column is evidence of the validity of the equation used. It is quite clear that the great variation in yields which is obtained is traceable to the climate of the area.

The differences between the items of the first column and those in the second represent the difference between the yields which are possible and those actually obtained. There are only two cases where agreement is good. In others, there is a considerable margin between what is produced and what might be expected. At Paia, Kihei, Upolu, and Halelua the departures are quite large. These four places are very windy. Crops on these fields invariably show low moisture levels or whipped leaves. Partial solution of the problem here appears to lie in the development of windbreaks. Although this is helpful, it is probable that planting the rows of cane closer together with a strong-topped variety will help to get the wind off the field.

TABLE IX

Estimated Yields at Several Locations for Two-Year Crops

Location	Normal Moisture, tons cane per acre	Actual Moisture, tons cane per acre
Ewa	122	127 (128)*
Waialua	120	95 (101)
Paia	127	97 (93)
Kihei	135	104 (99)
Upolu	124	100 (90)
Haleiua	106	78 (71)
Puakea	109	86 (86)
Maulili	79	57 (51)
Puuokumau	62	66 (50)

*Actual yield at each place appears in parentheses.

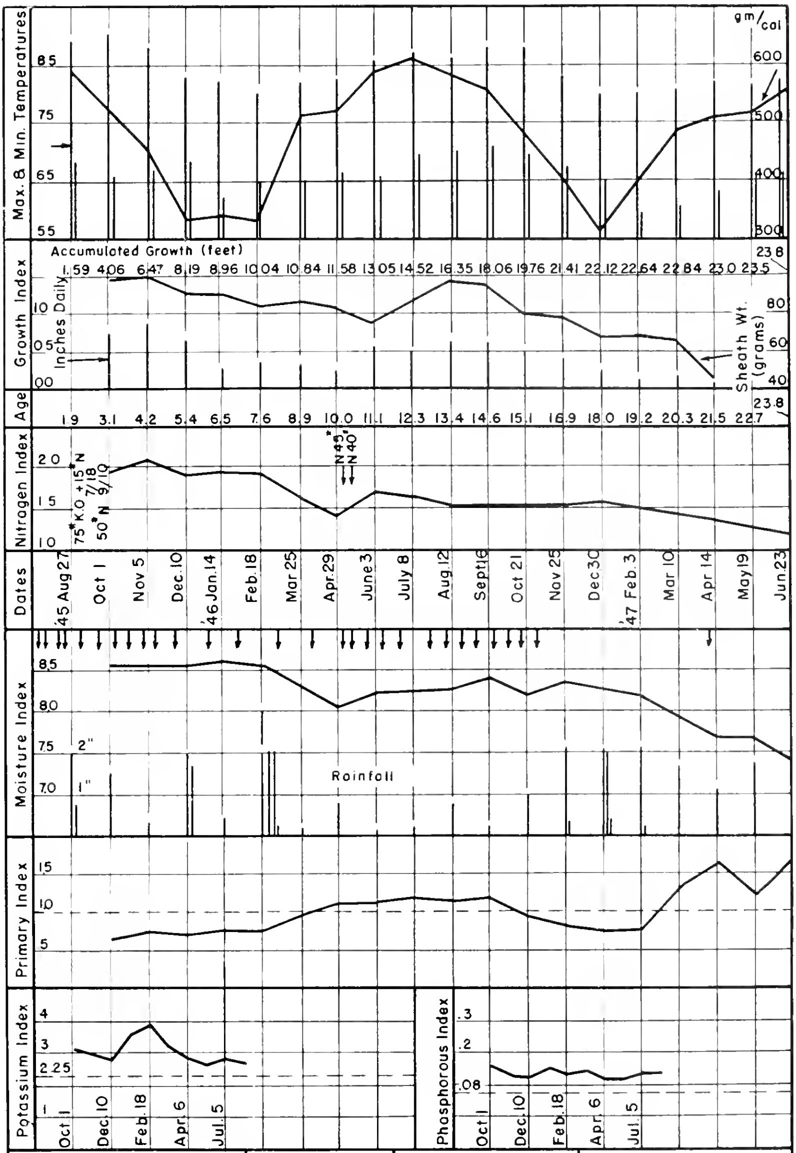
In some of these areas the soil is very compact and hard. In these areas no matter how frequent the irrigation, the moisture level remains low. Obviously, anything which will loosen these soils and improve their texture and permit a better penetration of roots and irrigation water will help raise the moisture level of the plants and thereby increase yields. In still others, such as Puuokumau, weed control has not been very successful.

I have said very little so far about the fertilizer requirements of the sugar cane crop. It should be very apparent to you that the amounts of mineral nutrients required by a crop are determined very largely by the climate in which the crop is growing. It seems only common sense that a crop growing in an area capable of producing 125 tons of cane per acre will require more plant food than one growing in an area capable of producing 50 tons per acre. The actual fertilizer applied will be the difference between that required by the plant in a given energy level and that available to the crop from the soil and irrigation water. It follows as a corollary that since climate is dominant in determining the quantity of growth obtained in the various areas of the islands, successive yields on a given field will also vary according to the weather actually experienced by each crop in the succession, and that, therefore, the fertilization of a given crop can reasonably be expected to vary from the previous crop. Furthermore, another variety of sugar cane can have a different requirement not only because its needs are different but also because its ability to extract the needed materials from the soil is different. With such a variety of circumstances affecting the welfare of a crop, it seems obvious to me that the well-being of a crop has to be followed while it grows, and that empirical practices at best can be successful only occasionally.

SUMMARY: THE CROP LOG

To bring into focus, then, the requirements of a crop as it grows so that it can be fed, and otherwise nurtured and guided to a successful harvest, the crop log (*1, 6, 2, 3*) was developed and is now in practical and successful use on over fifty thousand acres of sugar cane. Such a crop log is shown in Figure 1. It is a record of the crop's progress from its start until harvest and is made up of certain physical and chemical measurements and observations which serve as a guide to its handling.

In the top section is a record of the maximum and minimum temperatures and sunlight experienced by the crop as it grows. Below this is the Growth Index section. If elongation measurements are kept, they are recorded here. Accumulated growth is indicated on the top line. The green weight of the sheath sample per stalk is also recorded.



Crop Year	1947
Field	R 8
Acres	0.6
Cycle	1st Ratoon
Variety	32-8560
Elevation	20'
Soil Type	Residual

Started	6/29/45
Stand (6 mos)	Ex.
Harvested	6/24/47
Age	23.8
Trash %	
Dead Cane %	

HARVEST DATA

TCA	120.0	BRIX	21.0
TSA	15.58	Pol	18.7
TC/TS	7.70	Pur.	89.7
TCPAM	5.05		
TSPAM	.656		

FERTILIZATION, etc.

210 N o P_2O_5 73 K_2O
Age at 1st. N 0.63 mos.
Age last N 10.50 mos.
Date 1st. Irrig. 7/3/45
Total No. Rounds 31
Total Rainfall 31.58
Weed Control – Good
Tasselling 1st. Yr. – None
" 2nd. Yr. – V. Light

FIGURE 1. A completed crop log on sugar cane.

The nitrogen index which is the total organic nitrogen content of the green tissue of the leaf blades expressed in the dry-weight basis is plotted in the third section. Also along this curve are indicated the various applications of fertilizers made to the crop. At the top of this section is a record of the accumulating age of the crop.

At the bottom are the actual dates on which sample collections were made.

Below this is the section dealing with the moisture index. The actual tissue moisture levels are graphed. At the top, the downward pointing arrows indicate dates of irrigation rounds. At the bottom, the vertical bars indicate the rainfall received.

The Primary Index, or the total sugar level of the leaf sheaths is plotted next as percentage of the dry weight.

The potassium and phosphorus indices are shown next with the normal line dotted across the space at 2.25 and 0.080, respectively. The values are potassium and phosphorus contents expressed on the basis of the sugar-free dry weight.

At the bottom of the log are certain miscellaneous data which make the record for the crop complete. The log once completed becomes a permanent record for that crop. It has several uses: it is an excellent source of research material; it, along with others on the same field, serves to point up long-time trends; it is useful in comparing one field with another and often points up needs for differential preparation or cultivation. But its most important use is in guiding the current crop in its growth and culture to maturity.

To show its application I shall very briefly describe its use in handling a ratoon crop.

As soon as a field of cane is harvested and the cane lines are reshaped, the first irrigation water is applied. Fertilizer applications are then made. Nitrogen is always applied, and in high production areas 75 pounds of the element are put on. Potash is also applied at once if the previous crop log showed need for potash. If it did not, no potash is applied unless and until the potassium index drops below the normal line. Phosphates usually are not applied to ratoons but only to plant crops. During this early period of a ratoon, irrigations are based on a schedule. The tensiometer (9) is being used to excellent advantage here, irrigations generally going on with tensiometer readings of 0.30 atmosphere. This schedule is maintained throughout the growing cycle of the crop.

When the young crop is between two and three months of age, its plants are large enough to be sampled. Sampling is continued at 35-day intervals throughout the two years of the crop. From these samples, analyses are made for tissue moisture, nitrogen, potassium, phosphorus, and total sugars. These data are plotted on the log. If all our early operations, that is, weed control, irrigations, and fertilizations, have been well timed and properly done, the moisture index is above 85, the nitrogen index at or above 2 per cent, the primary index below 10 per cent, and the potassium and phosphorus indices above the normal line. These levels should be maintained throughout the first six months. A second application of 75 pounds of nitrogen is made at three to four months of age except in a few cases of high residual soil nitrogen. On these, the nitrogen index remains high and the second application is canceled. Occasionally, more nitrogen is applied if the index drops.

If all the indices are maintained at these desirable levels, the growth is excellent. Troubles may be encountered in maintaining the tissue moisture level. If the soil was compacted by the harvest, the moisture level of the next crop will drop even though irrigations are normal. In short, anything which interferes with proper growth of roots will result in the lowering of the moisture level and hence in the reduction of growth. Here we have a few things which can be done. Since nitrogen is a strong ally of moisture, we can apply small amounts of nitrogen repeatedly, we can run smaller streams of irrigation water down the furrows for a longer period of time, or we can insert trash dams in the furrows to effect better penetration of water. Actually, at best these are temporary expedients. It is usually best to correct such difficulties when the field is plowed and planted. Accurate layouts of irrigation furrows, incorporation of organic materials, and the selection of a vigorous rooted variety all help to keep the moisture level up on these soils. If the moisture level is low because of severe exposure to winds, the selection of proper varieties is a considerable help.

We know that maintaining the moisture level correctly results in the production of heavy tonnage. To be sure, if the moisture level is not maintained because of fertilizer deficiency, that would quickly be picked up on the log and applications would be made. If weed control gets out of hand, the moisture level also drops.

If the moisture level is correctly maintained through the first eight to ten months of growth along with a low total sugar level, the cane growth is very heavy and is, of course, likely to be succulent. Lodging of the cane in this condition will result in breakage and killing of stalks, hence, we impose a hardening on such fields. Irrigations are discontinued until the moisture index drops to about 77 per cent. During this period, not only are the stalks hardened, but their sugar content builds up. The stools and very likely also the roots become loaded with sugar. That is, we not only consolidate tonnage gains made to date, but we also prepare the roots for another spurt of growth.

When the mid-crop hardening is completed, irrigation is resumed on schedule. Usually the nitrogen index has dropped by this time and the remaining fertilizer to be applied is now calculated and applied. If the potassium index has fallen below the line, potash is also applied. Thus, fortified with carbohydrate material and now with moisture and needed fertilizers, the crop is off for its second season of growth. The moisture level rises to between 80 and 83 per cent. Usually following the lodging of stalks, if the second season fertilization and irrigations are properly timed, a heavy flush of suckers is set off adding to the stalk population in the field.

After twelve months no further fertilization is practiced even though deficiencies develop, but irrigations are kept on schedule. Up until the crop is 17 to 18 months of age, our chief concern is the piling up of tonnage. Except for the period of mid-crop hardening, we are not concerned with quality. However, beginning with seven months before harvest and continuing until harvest time, our concern is no longer one of producing tonnage but becomes one of producing quality (8). Thus, at seven months before harvest, every field is put on a weekly sampling basis and the data are plotted on a ripening log. We know that for best quality the tissue moisture level should drop gradually to a level of 72 to 73 per cent. We also have learned that this level must be approached gradually. If during the second season of growth the moisture level has been higher—that is, 82 to 83 per cent—the irrigations are discontinued, the tensiometers are removed from the field, and the sheath moisture is used as the guide to further irrigations. Such a crop would not be irrigated until the moisture level dropped to approximately 79 per cent. An irrigation would be applied and a new, lower level set up, say 76 per cent. Obviously, if the moisture level of a crop is low throughout the second season, this crop has been ripening already and would call for a much-reduced ripening period. In any event, when the final moisture level of 73 per cent is reached, the crop is harvested. While the moisture level is dropping, the nitrogen level drops and the primary index rises. However, the dominant criterion of ripened cane is the moisture level of 73 per cent. This must be arrived at gradually. Ripening is not effected if the crop is simply dried out.

Now, as should be the case, if the crop log enables us to follow the welfare of the crop, it should enable us to focus our attention on the needs of a crop, and therefore should result in increasing yields. In some cases we have saved substantial outlays in fertilizers. In other cases, however, we have used more fertilizer. The important thing, however, is that we have not only increased the tonnage of cane produced but have improved the quality of that cane with a resulting increase in tons of sugar produced per acre per month.

Finally, we are learning from the crop log that if we maintain the moisture levels where they should be through proper timing of irrigation as well as fertilization and weed control we approach more closely each time the maximum production possible for the atmospheric energy available to us.

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