Notes

1. Many authors, in fact the majority, reserve the name "neutrino" for the particle which accompanies the positive β-decay, and call "anti-neutrino" the particle which is emitted in the negative β-decay. This choice is opposite to ours and would result in giving the neutrino a right-handed spirality. Our choice was done solely for the convenience of introducing the neutrino before the anti-neutrino.
2. The uncharged π-meson has an even shorter lifetime, but it will not be considered here.
3. In order to make this difficult chain of reasoning as simple as possible, we have made the assumption that the two neutrinos emitted by the μ-meson are identical. Actually, it seems more probable that they are of different kinds, neutrino and antineutrino. Under these conditions, the same conclusion can be reached, but only in a more subtle way.

Outer Space in Plants

Some Possible Implications of the Concept

Paul J. Kramer

For many years, work on the absorption of ions by plants has been dominated by the assumption that ion accumulation is *the* important process in ion absorption. For readers who are not familiar with the terminology of this field, these processes may be defined as follows. *Absorption* is a general term referring to the entrance of a substance into cells, tissues, or organs by any mechanism such as diffusion, mass movement, or active transport. Accumulation is a special type of absorption involving entrance against a concentration gradient by active transport. Accumulation requires the expenditure of metabolic energy by the cells or tissues in which it occurs; other absorption mechanisms do not. Active transport refers to movement of substances against a concentration or activity gradient, in contrast to passive movement, by diffusion along an activity or concentration gradient. The mechanism of active transport is not fully understood as yet, although theories involving carrier systems are popular at present (*1*).

There is increasing evidence that ion accumulation in cells may be a subsidiary process of importance chiefly at the cellular level and that ion absorption and translocation in intact plants occur more or less independently of accumulation. This possibility has been greatly increased by the development of the concept of outer space or apparent free space. By outer space is meant that fraction of the tissue volume into and out of which ions can move freely by diffusion.

Volume and Location

Hope and Stevens (*2*) seem first to have studied quantitatively this space in roots. They observed that up to 13 percent of the volume of *Vicia faba* roots consisted of space into and out of which ions are free to diffuse and termed it "apparent free space" (often abbreviated AFS). Butler (*3*) established the existence of free space in wheat roots by several methods and found that it comprised 24.5 to 33.5 percent of the root volume. Epstein (*4*) found that passive, reversible diffusion of several ions occurs into and out of a space in barley roots that is equivalent to 23 percent of their volume. Following the terminology of Conway and Downey (*5*), who had previously observed a similar situation in yeast cells, Epstein (*4*) termed the fraction of the root volume that is reversibly accessible to ions by diffusion "outer space.' The fraction of the tissue in which ions are accumulated by an active transport system was termed "inner space." The existence of space in cells accessible to various solutes by diffusion has also been observed in bacteria (*6*), in yeast (*5*), and in kidney tissue (*7*).

Although Epstein did not identify outer space with any particular region of cells, Hope and Stevens (*2*) and Butler (*3*) assumed that it included both cell walls and cytoplasm. It would be difficult to account of the volume of outer space observed in roots by various workers without including at least part of the cytoplasm. This means that the differentially permeable membrane which controls accumulation of ions is the tonoplast or vacuolar membrane rather than the outer surface of the protoplast or plasmalemma, as is often supposed.

That diffusion of ions into the cytoplasm occurs is indicated also by other types of experiments such as those of Brooks (*8*), Hoagland and Broyer (*9*), and Sutcliffe (*10*). Hope and Robertson (*11*), after reviewing previous work, concluded that the vacuolar membrane, rather than the plasmalemma, is the principal membrane in cells that is impermeable to solutes. Thus, inclusion of at least a part of the cytoplasm in outer space seems highly probable, although it has not been proved. Some binding of ions occurs in the cytoplasm, and apparently mitochondria accumulate ions and ought therefore to be excluded from outer space.

Thus far outer space has been discussed only in connection with the absorption of ions by roots, but if it occurs in roots it almost certainly also occurs in stems, leaves, and other plant structures. Perhaps practically all of the water-permeable structure of plants can be regarded as outer space, except the vacuoles, mitochondria, and ion-binding sites in the cytoplasm. Intercellular spaces are not included, because they ordinarily are occupied by air. Regardless of exactly what is included in outer space, the existence of a considerable volume in plant tissues into and out of which ions can diffuse freely must have important effects on other plant processes besides salt absorption.

Aids in Explaining Diverse Phenomena

The concept of outer space makes it possible to explain a number of phenomena which are difficult to explain if it is assumed that most of the ions in plants move from vacuole to vacuole by active transport, or are accumulated in vacuoles behind differentially permeable membranes. Examples are the increased absorption of minerals accompanying the increased absorption of water, the wide variety of ions found in plants, the absorption of large molecules such as chelates and antibiotics, and the leaching of ions from leaves by rain.

Outer space provides a pathway by which ions may move from the soil solution to the leaves without passing through the vacuole of a single cell. Furthermore, according to this concept, a considerable fraction of the salt, and perhaps of other solutes, is not irreversibly accumulated in vacuoles, but occurs in outer space where it can move freely by diffusion, aided by cytoplasmic streaming, or can be carried by mass flow. All movement of materials in the xylem and probably

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all movement in the phloem can be regarded as occurring in outer space. Movement of materials through the symplast, as proposed by Arisz (*12*) also might be regarded as occurring in outer space.

It is much more reasonable to suppose that ions move across the cortical cells of roots by diffusion, aided by cytoplasmic streaming, or are carried by mass flow in the transpiration stream, than to suppose that they are successively accumulated and released by each of the numerous cells through which they must pass. Arisz (*12*) and Epstein (*4*) have pointed out that it is difficult to understand how each of the several cells of the root cortex can accumulate ions on the outer side and lose them on the inner side, until finally the ions are secreted into the xylem. The energy requirements for such movement from vacuole to vacuole would be extremely high. Broyer (*13*) found some evidence that in roots that are high in salt content, movement of ions may occur through the symplast without their entering cell vacuoles, and Wiebe and Kramer (*14*) found that absorption of ions and translocation to the shoots of intact plants seem to occur independently of accumulation.

Scott and Priestley (*15*) long ago suggested that the soil solution diffuses into the root cortex and that ions are absorbed from this solution by the cortical cells. They believed that diffusion beyond the cortical parenchyma was prevented by the suberized radial walls of the endodermis, but if ions can diffuse through the cytoplasm, then the endodermis is not the important barrier it formerly was supposed to be. Furthermore, much of the water and salt absorption of perennial plants probably occurs through roots in which secondary growth has resulted in the disappearance of the endodermis.

The existence of a considerable percentage of the root volume as space in which ions are free to move by diffusion or mass movement explains why increased uptake of water often results in increased uptake of salt. Obviously ions in outer space would tend to be moved more rapidly into the xylem by more rapid inward movement of water. How much of this movement occurs through the cell walls and how much through the cytoplasm is uncertain. Strugger (*16*) has long insisted that considerable movement of water and solutes occurs in the cell walls. This may be true, but the marked reduction in both water and ion movement which occurs in the presence of respiration inhibitors (*17*) suggests that at some point water and ions pass through protoplasm. In young roots this may be chiefly at the endodermis, and in older roots where the endodermis has disappeared it probably is at the cambium.

The concept of outer space aids in explaining ion uptake by transpiring plants, but it increases the difficulty of explaining how ions are accumulated in the xylem elements of slowly transpiring plants in sufficient concentration to cause the occurrence of root pressure. The Crafts-Broyer (*18*) theory of accumulation depends on accumulation in the cytoplasm of the surface cells and movement into the stele by diffusion aided by cytoplasmic streaming. This system cannot operate if ions can diffuse in and out of the cytoplasm, as must be the case if cytoplasm is included in outer space. At present there seems to be no satisfactory way of explaining salt accumulation in the xylem.

The concept of outer space is very useful in explaining why plants contain almost every ion found in their environment, although individual cells are highly selective with respect to the ions accumulated in their inner space. All of the solutes in the soil solution probably are carried throughout plants in the transpiration stream and occur in the outer space of roots, stems, and leaves, but the various ions are accumulated in the inner space or cell vacuoles in quite different proportions from those occurring in outer space; hence, the total amounts of various elements present vary widely in different species. Apparently, because it occurs through outer space, absorption is much less selective than accumulation, while accumulation is brought about by an active transport system that operates only on certain ions and therefore is highly selective.

Movement of solutes in free space probably also explains the absorption of large molecules such as those of iron chelates (*19*). It is very unlikely that such large molecules are accumulated in cell vacuoles, but it is quite possible that they move through outer space. Apparently, at least part of the movement of antibiotics also occurs in outer space. Crowdy *et al.* (*20*) reported that the uptake of griseofulvin by broad beans and tomatoes is proportional to the volume of water transpired by the plants.

The concept of outer space in roots as the chief pathway for entrance of ions requires less emphasis on the ion-exchange properties of root surfaces and soil particles. If it is assumed that the soil solution actually diffuses into the root, then ion-exchange properties of the soil are important only insofar as they affect the composition of the soil solution. The factors affecting absorption and accumulation operate at the surfaces of or within the cells of the roots, rather than at the outer surfaces of the roots, as often has been supposed.

Leaching

The assumption that a considerable amount of the salt in leaves occurs in a diffusible condition in outer space facilitates the explanation of losses of solutes from leaves by leaching. It has been reported by several workers (*21*, *22*) that considerable amounts of salt are leached from leaves by rain, sprinkling, or brief soaking in water. It is difficult to understand how this could occur if most of the salt were accumulated within impermeable membranes, but it is obvious that solutes occurring in a freely diffusible state in outer space can be leached out very easily. Apparently, some organic compounds can be leached out also, for Long *et al.* (*22*) found that several amino acids and large quantities of a galactan were leached out of leaves. It also has been suggested that excessive loss of carbohydrates by leaching might explain some instances of heavy losses of newly set fruit during prolonged rainy periods.

Ions moved to the leaves in the transpiration stream presumably occur chiefly in outer space, from which they are accumulated in the vacuoles of the leaf cells. If transpiration is rapid, there may be a tendency toward salt accumulation in the outer space of leaves, and there is some evidence that rapidly transpiring leaves often contain more salt than slowly transpiring leaves (*23*), but ordinarily the concentration does not become very high, because of removal by leaching and by retranslocation out of the leaves through the phloem. According to this view, the cells of the leaves accumulate ions in their inner space from outer space in the same manner that root cells accumulate ions from the soil solution that occurs in their outer space.

It is probable that outer space also plays an important part in the absorption and movement of solutes applied to the leaf surface, such as foliar sprays. It is unlikely that such solutes are first accumulated in the inner space of cells, then released and translocated to other parts of the plant. It is much more probable that the solutes diffuse into the aqueous phase of outer space and are then either moved out through the phloem or accumulated.

The presence of an appreciable fraction of the total volume of plants in which ions are free to move by diffusion raises questions concerning the methods used in analytic studies. For example, roots usually are rinsed thoroughly in water before the uptake of ions is determined by either chemical or radiochemical methods, but such washing obviously removes a large part of those ions that occur in outer space. Epstein (*4*) found that SO_4^{--}, PO_4^{---}, SeO_4^{--}, and Ca^{++} were easily washed from outer space, and Russell and Adams (*24*) found that considerable phosphate was removed from barley roots by washing prior to analysis.

Leaves often are washed to remove dust from their surfaces, but this may also remove considerable amounts of various solutes. Although Hammar (*25*) found that washing did not materially affect the chemical composition of pecan leaves, the results of leaching studies on other species suggest that considerable salt and even organic solutes might be removed by washing. This increases the difficulty of dealing with roots covered with soil and leaves covered with dust or spray residue, but the probability of significant leaching during washing cannot be evaded. Britten (*26*) recently reported that proline is washed out of *Escherichia coli* cells by washing in water or a dilute solution.

It would be interesting to know more about the relative physiological importance of the ions and other solutes in outer and inner space. It might be argued that those substances which occur in outer space are in more intimate contact with cytoplasm and are more important physiologically than the larger fraction which is accumulated in the vacuoles. The usual methods of analysis do not distinguish between the fractions in inner and outer space, but methods could be devised to estimate the two fractions. Such information might be more useful in studies of translocation and plant nutrition than information concerning the total amount of an element present in a plant.

Need for Correlation of Research

Failure of previous investigators to apply the concept of outer space to an explanation of the various phenomena discussed in this article illustrates a basic weakness in modern science. Too little attention is being given to the correlation and interpretation of separate pieces of research. Thousands of investigators are collecting data and publishing data, but very few are correlating and interpreting their results. Investigators working with bacteria, yeast, plant roots, and animal tissue have all observed similar phenomena, but little attempt has been made to draw any general conclusions from these observations. The investigators who first developed the concept of outer space in roots thought of it only in terms of salt absorption in roots, while the workers studying salt absorption through leaves, leaching of solutes out of leaves, or the relation between water and salt absorption were so concerned with their own particular problems that they failed to see the relationship between their work and that of other investigators. As a result, the broad implications of the concept of outer space have been overlooked by most physiologists.

It is possible that further research will result in revision of the rather simple concept of outer space or free space which is now held. Its potential importance in explaining several different phenomena certainly justifies additional research to gain a better understanding of it. If outer space occupies as large a volume in plants as now seems probable, considerable revision of our present explanations of ion absorption and translocation may be necessary.

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A. S. Pearse, Ecologist

Arthur Sperry Pearse, emeritus professor of zoology at Duke University, died 11 December 1956 in Durham, North Carolina, at the age of 79 years. He was born 15 March 1877, on the Pawnee Indian Reservation near Crete, Nebraska, where his father and mother managed a trading post. His boyhood was spent there and in Denver, Colorado. In growing up he experienced many colorful adventures in the rough life of the western mining camps and pioneer farms of the period, and throughout his life he, like Theodore Roosevelt, was an ardent exponent of the strenuous life.

From the very beginning he was intensely interested in natural history, and it is not surprising that he became an ecologist. He began collecting in these early years. In his autobiography, *Adventure: Trying To Be a Zoologist,* he writes, "As a boy I loved animals and everything concerning them. I was born to be a zoologist." Many events and many people were to influence his career. While attending high school at Beatrice, Nebraska, he studied chemistry and physics under Samuel Avery, who later became president of the University of Nebraska.

At the University of Nebraska he played football—the beginning of a lifelong love of athletics. Characteristically, he left the university in 1898 to serve as corporal with the 4th Nebraska Volunteer Infantry in the Spanish-American War, returning to receive the bachelor of science degree in 1900. While teaching high school at Omaha he worked for his master's degree under the direction