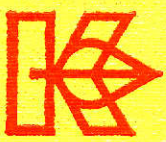
Potassium in Biochemistry and Physiology



International Potash Institute 1971

Potassium in Biochemistry and Physiology

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8th Colloquium of the International Potash Institute, Berne/Switzerland

June 14 to 17, 1971 in Uppsala/Sweden

High Patronage:	His Excellency, Mr. *I. Bengtsson*, Minister of Agriculture of Sweden
President of the International Potash Institute:	*P. Chaudet*, Former President of the Swiss Confederation
Chairman of the Colloquium:	Prof. *S.L. Jansson*, Head of the Department of Soil Fertility and Management, Agricultural College of Sweden, Uppsala/Sweden; Member of the Scientific Board of the International Potash Institute

Opening Address

P. CHAUDET, Former President of the Swiss Confederation, President of the International Potash Institute, Berne, Switzerland

Your Excellency,
Mr Dean of the Agricultural College of Sweden,
Ladies and Gentlemen,

I have the honour and the pleasure to welcome you at this 8th International Colloquium organized by the International Potash Institute. Let me first express my deep gratitude to His Excellency, Mr *Ingemund Bengtsson,* Minister of Agriculture of Sweden, for having accepted to assume the High Patronage of this scientific gathering. I want to express our lively appreciation and our great pleasure for having been able to hold in Sweden this Colloquium on Biochemistry and Physiology of Potassium in Plants and Animals.

I have to express also my thanks to Mr *Hjelm,* Dean of the Agricultural College of Sweden, allowing us to use the facilities of the Agricultural University in Uppsala. I should like to express my gratitude to Prof. *Jansson,* Head of Department of Soil Fertility and Management at the Agricultural College of Sweden and Member of the Scientific Board of IPI, who kindly accepted to be the Chairman of this Colloquium and who gave us his efficient support in organizing this Colloquium.

The bases of the programme for these three days were established by the 15 members of the Scientific Board – particularly by Prof. *Jansson* and Forstander *Dam Kofoed*/Denmark assisted by Prof. *Mengel* and Director *de Tarragon,* Scientific Secretaries of IPI – to whom I am extending my thanks. Finally, I want to express my gratitude to Mr *Lindén,* Scientist, working in the Department directed by Prof. *Jansson,* for his co-operation in setting up the arrangements for this scientific event together with the Directors and the Staff of the International Potash Institute.

Let me also welcome the representatives of the Agricultural Press of Sweden. Their presence is an encouraging sign for the interest which scientific research work encounters in the modern information media which are constituting the indispensable link between science and agricultural practice.

The organization of scientific Congresses and Colloquia on an international level constitutes one of the main activities of our Institute. These gatherings are planned in working cycles. The working cycle going from 1964 to 1966 was devoted to *'Potassium and the Quality of Agricultural Products'* and comprised three Colloquia (1964: Morat/Switzerland; 1965: Belgrade and Lisbon) and one general Congress (1966: Brussels). The last working cycle dealt with the problems of *'Role of Fertilization in the Intensification of Agricultural Production'*, and comprised three Colloquia (1967: Finland; 1968: Florence; 1969: Israel) and one general Congress (1970: Antibes/France).

The purposes of the present cycle, which begins with our Colloquium here in Sweden, are

to make the point of the present state of knowledge concerning fundamental potassium problems, to open new ways for research and to determine the implication and consequences of fundamental research for practical agriculture. As you know, the first event of this working cycle is devoted to *'Potassium in Biochemistry and Physiology'*, the next one will be devoted to *'Potassium in Soil'*, whereas the Colloquium in 1973 will deal with similar problems but in relation with tropical conditions. In a final Congress, the conclusions will be drawn from the latest basic research findings presented in the foregoing Colloquia as well as their implication on farm production.

In establishing the Programme, the Scientific Board was fully conscious that the Colloquium in Uppsala will constitute an experiment in so far as he has placed this event under the topic *'Interdisciplinary Exchange of Scientific Knowledge'*. It is hoped that the Colloquium will help to bridge the often existing gap between different sciences and will allow specialists of different fields and from different countries to get together. Keeping in mind the high competency and reputation of the scientists presenting lectures and who are working in different scientific fields, I am fully convinced that this Colloquium will engrave in the memories of all participants the souvenir of a very useful meeting.

I declare open the 1971 Colloquium in Uppsala!

1st Working Session

Potassium in Biochemistry and Physiology of Plants

Chairman of the Session:

Prof. Dr. *Dj. B. Jelenic*, Head of the Department of Agricultural Chemistry and Plant Physiology of the Faculty of Agriculture, University of Belgrade/ Yugoslavia; Member of the Scientific Board of the International Potash Institute

Potassium and its Role in Enzyme Activation

HAROLD J. EVANS, Ph.D. and ROBERT A. WILDES, Ph.D., Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon/U.S.A.

Summary

Potassium, or some other activator univalent cation, is a necessary cofactor for many of the enzyme catalyzed steps in metabolic pathways. We have reviewed the evidence for the necessity of univalent cations for the starch synthetase reaction and for the activity of a series of enzymic steps in protein synthesis. Of those univalent cations present in normal living tissues, potassium is the only cation that has the appropriate properties and that is present in cells in sufficient concentration to fulfill the univalent cation requirements of the great majority of those enzymes whose activities are dependent upon univalent cations.
In regard to the mechanism of potassium activation of enzymes we have concluded the following: (a) the subunit structure of some enzymes is dependent upon the type of univalent cation present, (b) the capacity of some enzymes to bind a particular coenzyme is univalent cation dependent, (c) univalent cations such as potassium may behave as allosteric effectors, (d) univalent cations may influence the conformation of some enzymes without causing gross changes in physical structure, (e) activator univalent cations may stabilize reaction intermediates during enzyme catalysis or influence rates of catalysis by mechanisms not yet fully understood. The interaction of univalent cations with certain antibiotics may serve as model systems for an understanding of the interaction of univalent cations with enzyme molecules.

1. *Introduction*

Potassium is an essential element for all living organisms. It is the most abundant cation in the tissues of higher plants, making up about 1.7 to 2.7 percent of the dry matter of normal leaves *(Evans* and *Sorger [13]).* Animals also need large quantities of K^+ as illustrated by the report that 3 to 4 gm of KCl are necessary for a daily diet of 2000 calories *(Hall [14]).* The K^+ requirement of organisms cannot be completely satisfied by any other alkali cation, although in some plant species beneficial effects of Na^+ and Rb^+ have been reported when the K^+ supply was limited.
The consequences of K^+ deficiency in organisms are numerous. Some consistent effects of K^+ insufficiency are as follows *(Evans* and *Sorger [13]):* (a) soluble carbohydrates and reducing sugars accumulate; (b) starch and glycogen syntheses are impaired; (c) amino acids accumulate; (d) protein synthesis is blocked; (e) the utilization of respiratory substrates is retarded; and (f) oxidative phosphorylation and photophosphorylation rates are decreased. It seems clear that K^+ has a profound influence on a whole series of different metabolic processes.
In a recent review, *Evans* and *Sorger [13]* pointed out that the activities of some fifty different important enzymes known to participate in a variety of metabolic processes were either completely dependent upon, or were stimulated by, K^+ or some other univalent cation. They concluded: (a) that a large number of apparently unrelated types of enzyme-catalyzed reactions were activated by K^+ or other univalent cations; (b) that those enzymes activated by K^+ also were usually activated by Rb^+ and NH_4^+, but were

activated little by Na^+ and not at all by Li^+; (c) that the few enzymes (associated with halophyles) that were activated primarily by Na^+ also were activated by Li^+, but that such enzymes were not functional in the presence of K^+, Rb^+, or NH_4^+. They further noted that the concentrations of univalent cations necessary for the activation of most of the enzymes were relatively high, ranging around 0.01 *M* or greater for operation at one-half maximum velocities and at least 0.05 *M* for maximum velocities. The concentration of K^+ necessary for activity of univalent-cation-activated enzymes was considered to be sufficient to account for the high concentrations of K^+ that are known to be essential for normal growth and development of organisms. *Evans* and *Sorger* [*13*] indicated that the major role of K^+ in cellular metabolism is that of an enzyme activator and they proposed that K^+ and similar univalent cations induced specific conformations of enzyme proteins that are necessary for catalytic activity.

In a recent review, *Suelter* [*64*] has reconsidered the role of univalent cations in enzyme activation, and has compiled a list of some 60 enzymes that appear to require univalent cations for activity. He has separated these into two main classes of reactions, one involving phosphoryl transfer and the second involving elimination reactions. *Suelter* concluded that the enzyme-catalyzed reactions that require univalent cations either involve, or are presumed to involve, keto-enol tautomers as intermediates. *Suelter* [*64*] postulated that univalent cations act in the formation of a bridge between the enzyme and the keto-enol reaction intermediate.

In this paper, we propose to summarize information on the role of K^+ in the syntheses of protein and of starch as two examples of major metabolic processes that require K^+. In addition, we propose to outline some of the recent results that we consider to be pertinent to an understanding of the mechanism of enzyme activation by K^+ and other univalent cations.

2. *Role of potassium in protein synthesis*

Considerable nutritional evidence indicates that K^+ deficiency in several different kinds of organisms results in impaired protein synthesis. Although such evidence suggests sites of metabolic blocks, the consequences of nutritional deficiency often are complex and thus, evidence suggesting a site of action of an element may be an indirect effect of some other metabolic lesion. Detailed biochemical investigations, along with nutritional research may provide more definitive information. Recent refinements in both physiological and biochemical investigation have provided an opportunity for considerable insight into the role of univalent cations in the protein synthesizing apparatus.

2.1 *Requirements for the synthesis of nitrate reductase*

As an approach to the problem of the role of K^+ in protein synthesis, *Nitsos* and *Evans* [*35*] have investigated the effects of K^+ deficiency on the inductive formation of nitrate reductase in *Neurospora crassa*. Since nitrate reductase is not synthesized by *Neurospora crassa* in a medium lacking nitrate, the fungus may be cultured in a K^+ deficient medium containing glutamate and the mycelia utilized to study the effects of univalent cations on the formation of nitrate reductase. Replicated portions of the K^+ deficient mycelia were transferred to a series of media all containing nitrate, but with a series of different

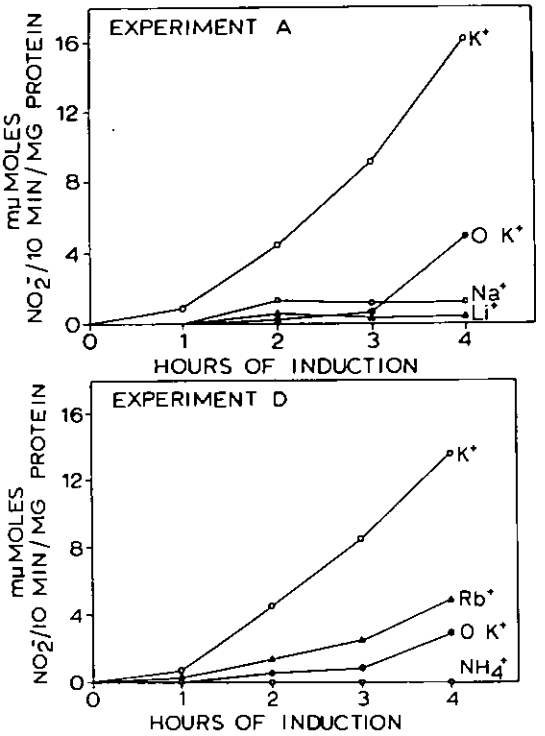

Figure 1. Representative experiments comparing the effectiveness of different univalent cations for the adaptive formation of nitrate reductase in *Neurospora crassa*. The mycelium was grown in a standard medium having no added K^+ and then transferred to the induction medium having a concentration of 2.5 m*M* of the indicated univalent cation chloride. (From *Nitsos* and *Evans* [*35*]).

univalent cation chloride additions. Each series of mycelial mats was incubated for a 4-hour induction period and the effects of variation in univalent cation additions on the formation of nitrate reductase determined. K^+ deficient mycelia exhibited no nitrate reductase activity at the beginning of the induction period (Figure 1). During the 4-hour incubation period the enzyme was rapidly formed in the medium containing 2.5 m*M* KCl. In contrast, where no KCl was added, or where 2.5 m*M* LiCl or NaCl was added, relatively little nitrate reductase was synthesized. In a similar experiment (Figure 1), RbCl (2.5 m*M*) proved to be about 50 percent as effective as KCl. NH_4Cl was completely ineffective as a cation cofactor in this system, but the NH_4^+ ion has been reported *(Sorger* [*59*]*)* to repress the synthesis of nitrate reductase. The induction of nitrate reductase in rice seedlings also is accelerated by univalent cations, and the same order of effectiveness as observed in *Neurospora crassa* was reported *(Oji* and *Izawa* [*37*]*)*.

In other experiments *(Nitsos* and *Evans* [*35*]) the effects of a series of univalent cations on the activity of pyruvate kinase, a constitutive enzyme in *Neurospora crassa,* were measured during a 4-hour period when nitrate reductase was being induced. The different univalent cation species had no consistant stimulatory effect on pyruvate kinase in these tests. It appeared, therefore, that univalent cations were influencing the synthesis of nitrate reductase rather than activities of enzymes in general.

Experiments that demonstrate an effect of univalent cations on the synthesis of a protein

such as nitrate reductase do not permit the identification of the precise sites where K^+ participates in protein synthesis. Such experiments, however, are relatively simple in design and have the unique advantage of indicating the univalent cation requirements for rapid synthesis of a single protein under *in vivo* conditions.

2.2 *Site of univalent cation action*

Relatively recent advances in our understanding of the detailed mechanism of protein synthesis have made it possible to obtain some fairly precise information on the specific biochemical sites where K^+ participates in the protein synthesizing process. Some of the sites in the protein synthesis pathway where K^+ has been proposed to function are illustrated in Figure 2.

2.2.1 *Synthesis of ribosomes*

In an investigation of the role of K^+ in protein synthesis, *Lubin* [*24*] used an *E. coli* mutant (strain B 207) that had lost its capacity to accumulate K^+ from the nutrient medium. This organism grew rapidly in a medium with high levels of K^+, but in a similar medium in which Na^+ was substituted for K^+, cell division stopped, and was not restored by the addition of amino acids, purines, pyrimidines, or vitamins. Further research *(Ennis* and *Lubin* [*11*]*)* revealed that K^+ deficiency in the mutant resulted in an accumulation of RNA, part of which was associated with 14S and 18S particles (K^+-depletion particles). When the deficient cells containing labeled 'K^+-depletion' particles were placed in a high K^+ medium that supported growth, the particles were converted into normal 30S and 50S ribosomes. *Ennis* and *Lubin* [*12*] indicated that the 'K^+-depletion' particles may contain the messenger RNA (mRNA) necessary for the synthesis of ribosomal protein. When K^+-deficient cells were transferred to a medium containing 0.1 *M* KCl, rapid and preferential synthesis of proteins necessary for formation of functional ribosomes was observed *(Ennis* and *Lubin* [*12*]*)*. A role of K^+ in ribosome synthesis seems to have been convincingly demonstrated.

The stability of ribosomes of the halophytic bacterium, *Halobacterium cutirubrum,* was reported *(Bayley* and *Kushner* [*3*]*)* to be dependent upon K^+ and Mg^{++}. This organism normally maintains high intracellular concentrations of salts and its ribosomes were maximally stable in solutions of 4M or greater KCl and 0.1 *M* $MgCl_2$. In media with low K^+ and Mg^{++}, or in media where K^+ was replaced by NH_4^+, Cs^+, or Na^+, the ribosomes dissociated into 30S and 50S subunits.

Evidence was presented *(Lubin* and *Ennis* [*25*]*)* that K^+ functioned in the formation of an effective polyribosome complex that apparently preceded the actual incorporation of amino acids into protein. More recently, *Ennis* [*10*] has provided convincing evidence of the role of K^+ in the formation of polyribosomes in *E. coli.* Polyribosomes disintegrated completely in cells depleted of K^+ and were found only in small numbers in cells grown in media containing low K^+ concentrations. Protein synthesis, however, exhibited a greater K^+ requirement than polyribosome formation. *Ennis* [*10*] concluded that protein synthesis was not limited by the polyribosome content during K^+ depletion.

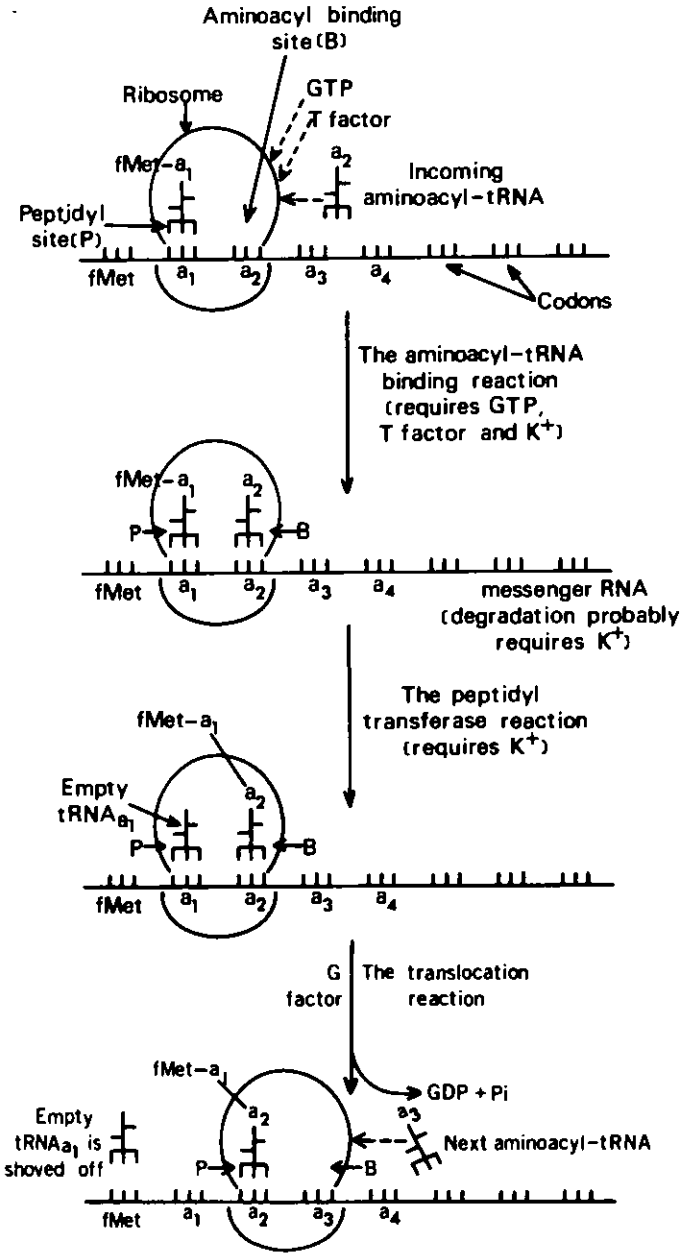

Figure 2. An illustration of the mechanism of the transfer of amino acids from aminoacyl-tRNA to the elongating peptide chain on the 70 S microbial ribosome. The T factor is a cytoplasmic protein necessary for the binding of aminoacyl-tRNA to the ribosome and the G factor is another cytoplasmic protein required for the translocation reaction.

2.2.2 *Amino acid incorporation into polypeptides*

2.2.2.1 Synthesis of aminoacyl-tRNA*

Schweet et al. [*53*] have shown that the tyrosinyl-tRNA synthetase from hog pancreas was much more active in a medium containing K^+ that in one containing Na^+. Also, the activities of leucyl-tRNA synthetase *(Yu* and *Hirsh* [*72*] and the lysyl-tRNA synthetase *(Waldenström* [*67*]*)*, both from *E. coli,* were reported to be stimulated by NH_4^+ and K^+. *Lubin* and *Ennis* [*25*] have investigated the possibility that K^+ deficiency in *E. coli* resulted in a lesion in the mechanism whereby tRNA is charged by amino acids. They [*25*] found that the substitution of K^+ for Na^+ in the tRNA charging reactions involving ^{14}C phenylalanine and other amino acids produced no more than 2- to 3-fold increases in activities. This magnitude of response in their opinion was not sufficient to explain the striking effects of K^+ additions on the rate of protein synthesis in the K^+ requiring *E. coli.*

2.2.2.2 *Protein synthesis from charged tRNA*

The requirement of K^+, or NH_4^+, for the transfer of amino acids from aminoacyl-tRNA to polypeptides has been firmly established in cell-free systems from several organisms (Figure 2). This process was studied *(Lubin* [*24*]*)* in a cell-free reaction containing appropriate cations, phenylalanyl-tRNA, poly U, GTP, ATP, buffer, and ribosomes and soluble enzymes from *E. coli.* In this system, the transfer of phenylalanine into polyphenylalanine proceeded at rates up to 20-fold greater in the presence of 0.05 M KCl than in a similar reaction containing NaCl (Figure 3). In these experiments NH_4^+ or Rb^+ substituted for K^+, but Li^+ and Na^+ were inactive.
Polyphenylalanine synthesis from ^{14}C-phenylalanyl-tRNA by a cell-free system from rabbit reticulocytes also depended on the K^+ concentration in the medium *(Shaeffer et al.* [*55*]*)*. Maximal activity occurred at 67 m*M* KCl and 6.7 m*M* $MgCl_2$. Also the transfer of amino acids from aminoacyl-tRNA to endogenous polypeptides in rat liver preparations required 80 m*M* NH_4^+ for optimum activity *(Skogerson* and *Moldave* [*57*]*)*. The evidence seems to be consistent that the protein synthesizing apparatus from both animals and microorganisms requires univalent cations for the incorporation of labeled aminoacyl-tRNA into protein.

2.2.2.2.1 Aminoacyl-TRNA binding to ribosomes

The step in protein synthesis where aminoacyl-tRNA is bound to ribosomes also is reported to require univalent cations (Figure 2). *Spyrides* [*63*] has studied the binding of ^{14}C-phenylalanyl-tRNA to *E. coli* ribosomes in the presence of poly U and observed that the reaction was stimulated markedly by NH_4^+ at a concentration of 0.16 to 0.2 *M*, and that K^+ at equivalent concentrations was about one-half as effective as NH_4^+. Both Na^+ and Li^+ were inactive in this reaction. The binding reaction did not require GTP or soluble transfer enzymes and, therefore, appeared to be non-enzymatic.
The binding of phenylalanyl-tRNA to reticulocyte ribosomes from the rabbit also marked-

* See: 'Abbreviations' page xxx.

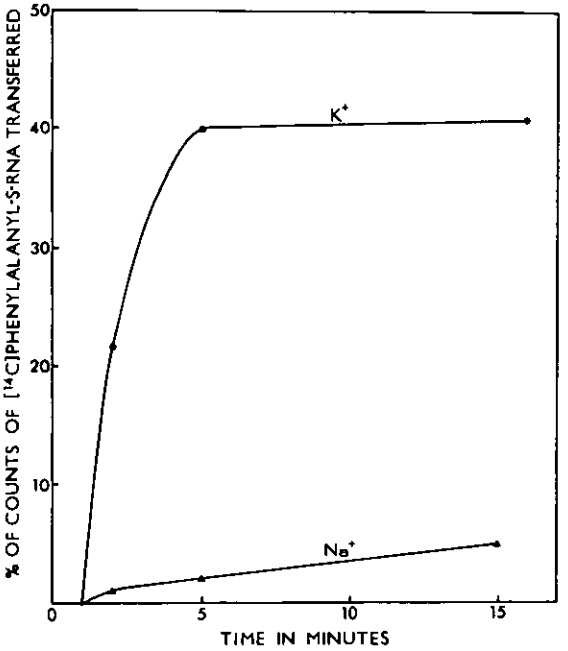

Figure 3. Kinetics of transfer of counts from L [C^{14}]phenylalanyl-tRNA to TCA-precipitable polypeptide at 37°C. Each milliliter of reaction mixture contained: 100 μmoles Tris-HCl (pH 7.4); 13 μmoles magnesium acetate; 9 μmole mercaptoethanol; 50 μmoles sucrose; 0.03 μmole trisodium-GTP; 1.0 μmole disodium-ATP; 6 μmoles disodium creatine phosphate; 20 μg creatine phosphokinase; 80 μg polyuridylic acid; L-[C^{14}]phenylalanyl-tRNA (0.0075 μc, specific activity 210 μc/μmole phenylalanine); S-30 fraction (4 mg protein); and 50 moles of either KCl or NaCl. (From *Lubin* and *Ennis* [*25*]).

ly responded to K^+ additions *(Shaeffer et al. [55)*. In these experiments enzymic binding of phenylalanyl-^{3}H-tRNA to the reticulocyte ribosomes in the presence of poly U required GTP and a soluble transfer enzyme, and occurred at K^+ and Mg^{++} concentrations that were optimal for the synthesis of polyphenylalanine (6.7 m*M* $MgCl_2$ and 67 m*M* KCl). When the $MgCl_2$ concentration was greater, and the KCl concentration less than 6.7 and 67 m*M* respectively, non-enzymic binding was observed that did not require GTP or the transfer enzyme. The most effective univalent cation for both enzymic and non-enzymic binding was K^+. NH_4^+ was one-third as effective as K^+, but neither Na^+ nor Li^+ were functional in this system. *Shaeffer et al.* [*55*] proposed that the molar ratio of KCl to $MgCl_2$ may influence the type of aminoacyl-tRNA binding to the ribosomes by altering ribosomal conformation. This conclusion is consistent with that of *Schweet et al.* [*52*], who also proposed that K^+, or NH_4^+, and divalent cations played an important role in protein synthesis by reticulocytes by influencing shape changes in the ribosomal structure. Experimental results by *Ibuki* and *Moldave* [*20*] in which ribosomes from rat liver were used and by *Ravel et al.* [*44*], who studied the binding of phenylalanyl-tRNA to *E. coli* ribosomes are generally consistent with those obtained by *Spyrides* [*63*] and *Shaeffer et al.* [*55*]. It seems clear, therefore, that univalent cations play some unidentified role in the binding of aminoacyl-tRNA to ribosomes.

2.2.2.2.2 Peptidyl transfer

By the use of puromycin as an inhibitor of protein synthesis evidence has been obtained that the peptidyl transferase reaction (Figure 2) is univalent cation dependent. Puromycin is an analogue of aminoacyl-tRNA and forms a peptide bond with the peptidyl-tRNA that is bound to ribosomes. The resulting peptidyl-puromycin complex, which lacks the nucleotide portion of aminoacyl-tRNA, is released from the ribosomes without further reaction *(Heintz et al. [16])*. *Maden* and *Monroe* [*26*] have demonstrated that both univalent and divalent cations were required for the reaction between phenylalanyl-tRNA charged ribosomes (from *E. coli*) and puromycin. The order of effectiveness of cations was $NH_4^+ > K^+ = Rb^+ > Cs^+$. Activation by NH_4^+ and K^+ progressively increased between 10 m*M* to 1 *M*, whereas, Na^+ and Li^+ at comparable concentrations failed to activate. *Maden* and *Monroe* [*26*] favor a model in which there is an interaction between univalent cations and one or more protein components of the 50S ribosome subunit which induces at the catalytic center a conformational change that is necessary for both peptidyl transfer and puromycin binding.
Skogerson and *Moldave* [*57*] have demonstrated that NH_4^+ was required for the reaction of puromycin with endogenous peptidyl-tRNA bound to rat liver ribosomes. The reaction was stimulated 5-fold by 300 m*M* NH_4Cl. The formation of diphenylalanine from reticulocyte ribosome-bound phenylalanyl-tRNA and phenylalanine in the presence of peptide synthetase from reticulocytes was stimulated 2-fold by KCl or NH_4Cl at a concentration of 67 m*M* *(Shaeffer et al. [55])*. There seems to be general agreement that peptidyl transfer is another site in protein synthesis where univalent cations participate.

2.2.2.3 GTP utilization

In the diagram (Figure 2) GTP is utilized in the aminoacyl-tRNA binding reaction and in the translocation reaction of protein synthesis by *E. coli*. Also, as discussed by *Boulter* [*6*] GTP is utilized in the initiation reaction in which formyl methionyl-tRNA is bound to the formyl methionine site of mRNA. In all three sites where GTP is utilized the products are GDP and inorganic phosphate. *Conway* and *Lipman* [*7*] have studied the relationship between polyphenylalanine synthesis (in a system containing poly U, ^{14}C phenylalanyl-tRNA and enzymes and ribosomes from *E. coli*) and the associated GTPase activity. In this study both the polyphenylalanine synthesizing system and the associated GTPase, required either NH_4^+ or K^+, but failed to function in the presence of Na^+ or Li^+. From the information available it is not clear whether the univalent cation requirement was associated with the GTPase *per se* or whether the association of GTPase activity and univalent cation content is an indirect consequence of some other univalent-cation-requiring step in the protein synthesizing process.

2.2.3 *Messenger RNA turnover*

The degradation of mRNA from the ribosomes after protein synthesis requires RNA depolymerising enzymes. The properties of K^+-activated phosphodiesterases have been investigated by several workers *(Singer* and *Tolbert* [*56*]*)*, *(Spahr* and *Schlessinger* [*62*]*)*, *(Natori* and *Mizuno* [*33*]*)*, *(Spahr* [*61*]*)*, and the possibility that enzymes of this type play

a role in mRNA breakdown and turnover has gained increasing support. In a thorough study of the properties of K^+-activated phosphodiesterase from *E. coli* (RNase II), *Spahr* [*61*] showed that the activity of the enzyme on polyuridylic acid was predominantly exonucleolytic and that both K^+ and NH_4^+ functioned as activators, whereas, neither Li^+ nor Na^+ was effective. Maximum response to K^+ or NH_4^+ was obtained at a concentration of about 0.05 *M*. The properties of a K^+-activated phosphodiesterase isolated from *Salmonella typhimurium* by *Ray* and *Burma* [*45*] were similar to those described for RNase II from *E. coli (Spahr* [*61*]*)*.

Natori and *Mizuno* [*33*] have investigated the degradation of rRNA and mRNA in wild-type *E. coli* and a mutant lacking RNase I. The pattern of degradation of rRNA and mRNA was similar in both organisms. The reaction proceeded much more rapidly in a medium containing K^+ than in a medium containing Na^+. *Natori* and *Mizuno* concluded that RNase II was involved in the degradation of mRNA in both *E. coli* strains.

Barnard [*2*] recently reviewed the evidence implicating RNase II in mRNA degradation in *E. coli* and pointed out that the enzyme exhibits specificity for single stranded RNA, and that the main action of the enzyme is exonucleolytic. Under *in vivo* conditions, therefore, when mRNA is associated with DNA, or when it is bound to ribosomes, presumably it would not be susceptable to attack. Messenger RNA would be susceptable to the phosphodiesterase, however, during that phase of protein synthesis where mRNA is detached from polysomes. There appears to be sufficient circumstantial evidence to strongly implicate a role for K^+-activated phosphodiesterases in mRNA turnover during protein synthesis.

3. *Role of potassium in starch synthesis*

Although it is well known that many starch-producing higher plant species respond to unusually high application of K^+ fertilizer, and that K^+ deficiency in many species results in decreased starch contents *(Evans* and *Sorger* [*13*]*)*, the role of K^+ in the starch synthetase reaction was not realized until recently. In 1966, *Akatsuka* and *Nelson* [*1*] observed that the addition of K^+ salts stimulated starch synthetase activities of extracts of immature maize seeds. The greater response was obtained when adenosine diphosphate glucose (ADPG) was used as a substrate. Although no absolute K^+ requirement was demonstrated, and no effort was made to eliminate K^+ from the assay reagents, the results clearly indicate that K^+ increased starch synthetase activity and protected the enzyme from thermal inactivation. *Murata* and *Akazawa* [*29*] and *Nitsos* and *Evans* [*36*] in 1968 and 1969 have provided more definitive information on the role of K^+ in the starch synthetase reaction. The findings of these two groups in general are in good aggreement. In this discussion, however, it is convenient to consider in more detail some of the results from our own laboratory.

The ADPG-starch transglucosylase (starch synthetase) reaction may be illustrated as follows:

$$\text{ADP-glucose} + \text{starch} \longleftrightarrow \text{ADP} + \alpha(1\text{—}4)\,\text{glucosyl} - \text{starch}$$

The assay for starch synthetase routinely used by us involved a coupled reaction that permitted the measurement of ADPG-dependent formation of ADP in presence of starch synthetase and endogenous starch primer. After incubation of the reaction containing particulate starch synthetase, ADPG, buffer, and the appropriate univalent cation salt, the reaction was terminated by heating. Then the ADP was assayed by the use of the pyruvate

kinase reaction which consumes ADP and produces pyruvate from phosphoenolpyruvate. The validity of this assay was established by use of ^{14}C glucose labeled ADPG, and measurement of the rate of incorporation of radioactivity into starch. Granules containing particulate starch synthetase and endogenous starch were prepared from seeds by an acetone precipitation procedure, and were stored frozen. Before use the granules were washed (suspended and centrifuged) at least three times in 0.1 *M* Tricine buffer, pH 8, in order to remove most of the endogenous univalent cations. Commercial preparations of ADPG or UDPG were converted to Tris-salts before use in the starch synthetase reactions. The use of appropriate precautions to minimize alkali univalent cation contents of reactions made it possible to obtain striking responses to univalent cation additions.

As illustrated (Figure 4), the rate of starch synthesis in a reaction catalyzed by a preparation from sweet corn was strikingly influenced by chloride salts of K^+, Rb^+, Cs^+, or NH_4^+. The addition of 0.05 *M* LiCl to a comparable reaction resulted in little activation, whereas, 0.05 *M* NaCl produced small but reproducible stimulations. From these results it seems obvious that starch synthetase from sweet corn is absolutely dependent upon univalent cations, and that K^+ is the most effective.

An examination of starch synthetase preparations from a variety of plant sources, including peas, soybeans, wheat, bush beans, and potatoes, revealed in every case a striking response, but not an absolute univalent requirement (Table 1). Analysis of the enzyme preparations from these species, however, indicated that the activities obtained without univalent cation additions could be accounted for by the endogenous univalent cation contents of the starch synthetase preparations. According to *Murata* and *Akazawa* [*30*] the addition of K^+ salts to reactions containing starch synthetase from sweet potato roots, white potato tubers, taro tubers, rice seed, barley seed and broad bean seed were stimulated markedly by KCl. In other experiments *(Nitsos* and *Evans* [*36*]*)* the type of anion added to starch synthetase reactions along with K^+ had no marked effect on activity and the substitution of divalent cations for K^+ resulted in an inactive system.

Although one would expect that the synthesis of starch and glycogen would involve similar mechanisms, the experiments of *Nitsos* and *Evans* [*36*] have shown no specific effect of univalent cation salts on the synthesis of glycogen in reactions containing UDGP and a glycogen synthetase preparation from rat livers. It is of interest that glycogen synthesis by animal preparations requires glucose-6-phosphate as a cofactor and does not respond to univalent cations, whereas starch synthetase from plants requires univalent cations and apparently does not involve glucose-6-phosphate. Since the starch synthetase system from higher plants is particulate and is not easily solublized and purified, no progress has been made in elucidating the mechanism whereby K^+ plays its important role in this reaction.

4. *Mechanism of potassium activation*

Although the effects of univalent cations on enzyme catalysis are striking it has been difficult to associate catalytic effectiveness with physical effects of univalent cations on enzyme proteins. The physical properties of some enzymes are markedly influenced by univalent cation additions while other univalent cation requiring enzymes exhibit no consistent physical alterations that are detectable by usual methods such as ultracentrifugation and ordinary electrophoresis. Sufficient evidence has accumulated, however, showing that univalent cations may strikingly alter some physical properties of certain enzyme proteins. Experiments in which effects are determined of univalent cations on the

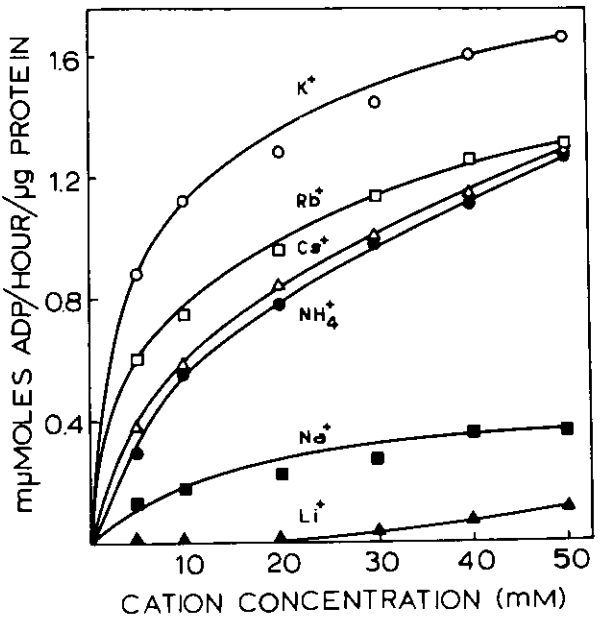

Figure 4. The effects of univalent cation chlorides on the activity of starch synthetase from sweet corn. Each reaction mixture contained 10 μmoles Tricine buffer, pH 8.0; 1.0 μmole Tris:ADPG; 4 mg starch granules (20 μg protein); and the indicated univalent cation chloride concentration in a total volume of 0.2 ml. (From *Nitsos* and *Evans* [36]).

Table 1. The effects of potassium on starch synthetase activity from different plant sources*

Source of enzyme	KCl concentration (m*M*)						
	0	5	10	25	50	75	100
	nmoles ADP/hour/μg protein						
Sweet corn	0.0	3.8	4.6	5.4	6.5	6.9	7.1
Peas	0.6	2.9	3.5	4.8	5.6	6.8	7.3
Soybeans	0.7	3.6	5.7	9.0	10.0	12.4	13.1
Field corn	0.9	5.5	6.8	7.6	9.0	9.4	9.8
Wheat	2.0	4.5	6.0	6.8	7.2	7.4	7.5
Bush beans	2.1	5.0	7.2	7.4	7.9	8.3	8.3
Potato	2.8	6.1	9.8	12.6	15.2	17.4	17.4

* The reaction mixture contained the following components in a total volume of 0.2 ml; 10 μmoles Tricine buffer, pH 8.0; 1.0 μmole ADPG, either 4 mg starch granules from bush bean seed, potalo tubers, wheat seed, field corn seed, pea seed, sweet corn seed or 2 mg starch granules from soybean seed and the indicated univalent cation concentration (From *Nitsos* and *Evans [36]*).

physical properties of enzyme proteins *per se* would not be expected to detect effects of univalent cations on the stabilization of intermediates of enzyme catalyzed reactions as suggested by *Suelter* [*64*].

4.1 Effects of univalent cations on physical properties

4.1.1 Immunoelectrophoresis and electrophoresis experiments

Sorger et al. [*60*] in 1965 reported that the immunoelectrophoretic pattern of a crystalline preparation of pyruvate kinase was markedly affected by the type of univalent cation present in the environment in which the immunoelectrophoresis was conducted. In the presence of a buffered medium containing activator cations such as K^+, Rb^+ or Na^+, the enzyme interacted with its antibodies after electrophoresis yielding a simple pattern that was detected by staining. In contrast, when the immunoelectrophoresis experiments were conducted in a comparable environment with the exception that non-activator univalent cations such as $Tris^+$ or Li^+ were added, distinctly more complex patterns of interaction of the enzyme with its antibody were exhibited. By shifting the cation environment from inactivator to activator univalent cations in these experiments, the non-activator-cation form of the enzyme was reversibly converted to the activator form. In control experiments, antibodies were prepared to highly purified catalase, an enzyme that does not respond to univalent cations, and the effects of univalent cations on the immunoelectrophoretic patterns determined. The interaction of catalase with its antibodies in these experiments was not influenced by the type of univalent cations in the immunoelectrophoresis environment. From these results it was concluded that activator univalent cations such as K^+, Rb^+, and Na^+ induced an active conformation of pyruvate kinase, and that non-activator univalent cations such as $Tris^+$ or Li^+ favored a non-active form of the enzyme. The recent results of *Reuben* and *Cohn* [*46*] confirm this interpretation.

Betts and *Evans* [*5*] have studied the effect of different univalent cations on the electrophoretic mobility of crystalline pyruvate kinase. The mobility of the enzyme in a solution containing weakly buffered Tris and 0.1 *M* KCl was compared with that in a similar solution with the exception that 0.1 *M* LiCl was substituted for KCl. Under these conditions, the electrophoretic mobilities were not influenced differentially by these two cations. Similar experiments were conducted with the exception that 0.01 *M* $MgCl_2$ was added to the enzyme solutions in KCl and in LiCl. In this case the electrophoretic mobility was consistently decreased in the solution containing Li^+. Effects on mobility of K^+ vs Li^+ also were detected in the presence of ADP and PEP, but the binding of substrates to the enzyme was not differentially influenced by these cations. These results are difficult to interpret because the addition of $MgCl_2$ to solutions of enzyme in KCl and in LiCl may differentially influence binding of K^+ and Li^+ and, thus, the observed effects may be indirect.

4.1.2. Spectrophotometric and circular dichroism investigations

In a series of investigations with pyruvate kinase from rabbit muscle, *Wilson et al.* [*71*] have observed that the enzyme was relatively stable in an environment where K^+, Rb^+ or NH_4^+ predominated but was relatively unstable when kept in solutions of $Tris^+$ or Li^+.

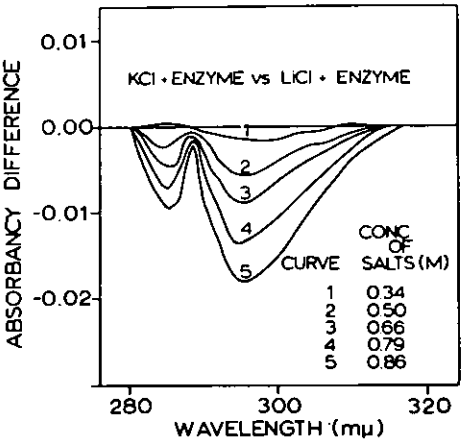

Figure 5. The effect of KCl as compared with LiCl at a series of concentrations on the difference spectra of pyruvate kinase. Each 3-ml mixture initially contained 0.033 M Tricine (pH 7.4), 6.7×10^{-4} *M* mercaptoethanol, 6 mg of pyruvate kinase, and concentrations of KCl (sample cuvette) and LiCl (reference cuvette) as indicated. The addition of increments of a concentrated solution of KCl or LiCl to attain the final molarities of salts indicated resulted in a dilution of the initial concentrations of buffer, mercaptoethanol, and enzyme by 0, 6, 12, 17, and 19% for reactions represented by Curves 1, 2, 3, 4, and 5, respectively. When the enzyme was omitted from both cuvettes the spectrum due to the different salts was not appreciably different from the base line. (From *Wilson et al* [71].)

These data indicated that stability of pyruvate kinase was associated with activator cations, whereas, instability was associated with non-activator univalent cations. These observations prompted a study of the effects of activator vs non-activator cations on the ultraviolet difference spectrum of the enzyme. A comparison of the enzyme in KCl vs enzyme in TrisCl revealed a striking difference spectrum. From further investigations it was concluded that $Tris^+$ was peculiar in that it induced perturbation of tryptophan residues, which resulted in marked absorption changes at 286 and 295 mμ.

In other experiments the effects of Li^+ vs K^+ at concentrations of 0.1 *M* or less produced no consistent evidence of ultraviolet perturbation. A comparison of the effects of these two cations at concentrations ranging above 0.3 *M* revealed marked spectral changes in the region near 286 and 295 mμ (Figure 5). The intensities of the absorption differences at these wavelengths were positively correlated with the concentrations of K^+ and Li^+ in the enzyme solution. Since the extent of perturbation appeared to be proportional to salt concentration it seemed reasonable to infer that the tryptophan residues of pyruvate kinase may be perturbed by 0.1 *M* or lower concentrations of Li^+ even though no such perturbation could be detected by the methodology employed. *Wilson and Evans* [70] concluded that the capacities of cations to perturb pyruvate kinase at relatively high concentrations were inversely correlated with capacities of univalent cations to function as enzyme activators. Furthermore, they emphasized that the effects of univalent cations on enzyme stability were negatively correlated with capacities of univalent cations to induce ultraviolet perturbation.

Wildes et al. [69] have used circular dichroism to investigate the effects of univalent cations on the structure of crystalline pyruvate kinase. No effects of univalent cations on the helical structure of the protein were revealed. In contrast, 0.1 *M* KCl or LiCl caused significant increases in elipticity in the 250 to 290 mμ range. These changes in the CD

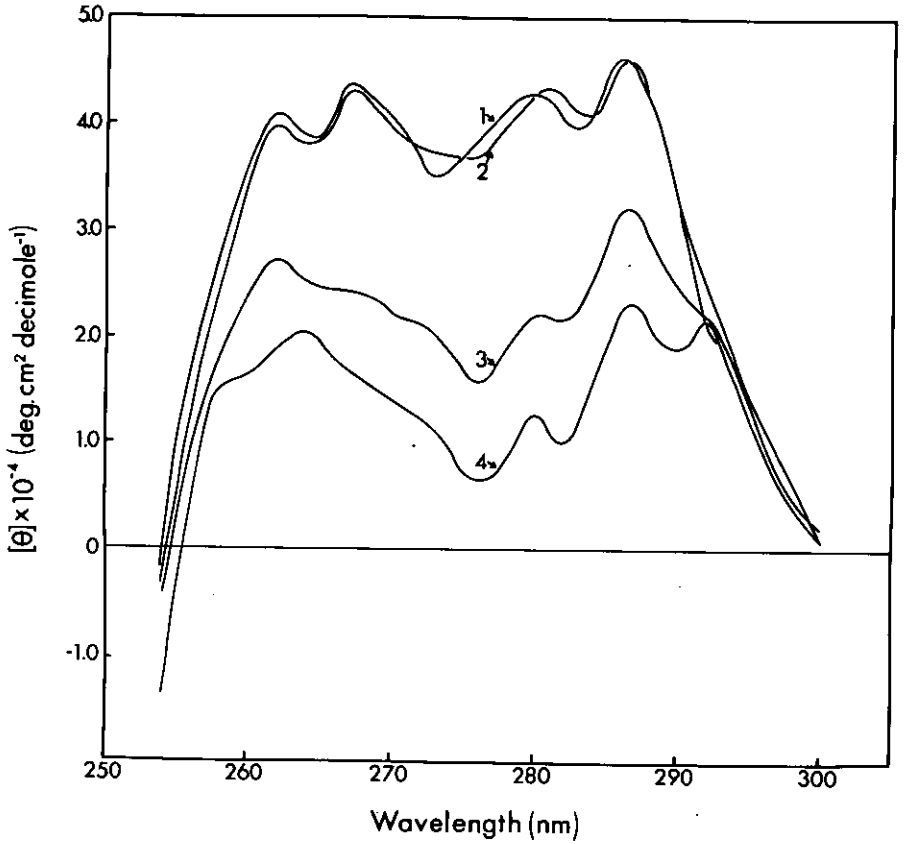

Figure 6. The effect of 0.1 *M* univalent cation chlorides on the CD spectrum of pyruvate kinase at 25 °C and pH 7.4. Curve 1, KCl; Curve 2, LiCl; Curve 3, TMACl; Curve 4, no added cations. Solvent: 0.01 *M* Tricine, 0.001 *M* 2-mercaptoethanol. The protein concentration was 2.9 mg per ml and the optical path length was 1.0 cm. (From *Wildes et al.* [69]).

spectrum occurred both in the absence and the presence of $MgCl_2$ and phosphoenol pyruvate. In control experiments 0.1 *M* tetramethylammonium (TMA^+), an ion that fails to activate pyruvate kinase, induced very little change in the CD spectrum. In Figure 6 the CD bands in spectra 1 and 2 at 262 and 268 mμ, and in spectrum 4 at 258 and 264 mμ, correspond closely to the CD bands produced by model phenylalanine compounds, and by phenylalanine residues of peroxidase. It appears, therefore, that KCl or LiCl additions to pyruvate kinase results in an alteration of the asymmetric environment of phenylalanine residues. The increase in ellipticity at 280 mμ also suggested that the presence of KCl and LiCl altered the asymmetric environment of tyrosine residues. Although these results are interesting, they are difficult to relate to physiological roles of ions since K^+, an activator, and Li^+ a nonactivator, of pyruvate kinase behave similarly. These results, however, are consistent with the observations of *Betts* and *Evans* [5] indicating that the extent of binding of substrates to pyruvate kinase were not differentially influenced by Li^+ and K^+.

4.1.3. *Magnetic resonance studies*

Mildvan and *Cohn* [*27, 28*] have obtained information on the mechanism of pyruvate kinase reaction from experiments in which magnetic resonance techniques were used to investigate the interaction of the enzyme and substrates with the paramagnetic Mn^{++} ion. Both Mn^{++} and Mg^{++} are activators of pyruvate kinase and are bound at the active site. The relaxation rate of water protons (PRR), as determined by nuclear magnetic resonance, was enhanced when Mn^{++} formed binary and ternary complexes with pyruvate kinase and the substrates ADP, ATP, and pyruvate in the presence of K^+ *(Mildvan* and *Cohn* [*27*]*)*. When KCl was replaced with TMACl, a non-activating univalent cation salt, the enhancement of PRR in the presence of the ternary complex of enzyme, Mn^{++}, and substrate was altered, indicating a change in conformation. *Mildvan* and *Cohn* [*27*] concluded that K^+ was necessary to maintain the correct conformation of the ternary complexes.

Reuben and *Cohn* [*46*] have obtained additional information on the binding of Mn^{++} to pyruvate kinase by use of electron paramagnetic resonance measurements. They concluded that a maximum of four Mn^{++} ions were bound to each pyruvate kinase molecule, and that the number of Mn^{++} ions bound decreased as the temperature was lowered from 37 °C to 5 °C. These findings were interpreted to mean that the enzyme existed in two conformations, one of which was active when four Mn^{++} ions were bound and the second of which bound no Mn^{++} and was inactive. The equilibrium between these two forms was found to depend on the ionic environment. In the presence of K^+ the equilibrium favored the active form, but in the presence of TMA^+ the equilibrium favored the inactive form. The conclusion of *Reuben* and *Cohn* [*46*] that K^+ stabilized the active form of the enzyme confirms the results of *Sorger et al.* [*60*], which was published in 1965 but not cited by *Reuben* and *Cohn* [*46*].

4.1.4. *Stability of the active enzyme form*

In certain univalent-cation-activated enzyme systems univalent cations play an important role in maintaining the stability of the active holoenzyme. Univalent cations may contribute to the functional integrity of enzymes of this type by stabilization of the subunit structure of the apoenzyme, or by facilitating the formation of an active holoenzyme from the apoenzyme and coenzyme.

Glycerol dehydrase from *Aerobacter aerogenes* catalyzes the conversion of glycerol to β-hydroxypropionaldehyde and activity is dependent upon the presence of coenzyme B_{12} and a univalent cation such as K^+, Rb^+, or NH_4^+. Na^+ and Li^+ are inactive in this system. The glycerol dehydrase apoenzyme is composed of two protein subunits that individually are catalytically inactive *(Schneider et al.* [*51*]*)*. Resolution of the two subunits was accomplished by passing the apoenzyme through a Sephadex G-100 column equilibrated with the non-activating cations Na^+ or cyclohexylammonium$^+$. In contrast, when the apoenzyme was passed through a similar column equilibrated with the activating cations, K^+ or NH_4^+, the apoenzyme emerged from the column intact and catalytic activity was retained. A functional apoenzyme was reconstituted from the separate subunits in the presence of K^+. In the presence of Na^+ and the substrate, glycerol, the apoenzyme remained intact but was inactive. It appears that the role of K^+ in the glycerol dehydrase reaction is not limited to that of maintenance of structural integrity of the apoenzyme but, in addition, is required for activity of the intact apoenzyme.

Formyltetrahydrofolate synthetase from *Clostridium cylindrosporum* has been reported to be fully active in the presence of NH_4^+, K^+, or Rb^+; partly active in the presence of Cs^+; and essentially inactive in the presence of Na^+, Li^+ or $Tris^+$ *(Himes* and *Wilder [17])*. By use of ultracentrifugation techniques *Scott* and *Rabinowitz [54]* have determined the effects of univalent cations on the stability of formyltetrahydrofolate synthetase In the presence of 50 m*M* K^+, NH_4^+, or Rb^+, the enzyme was stable and remained active, but in the presence of 50 m*M* Na^+, Li^+, Cs^+, or $Tris^+$, the enzyme dissociated into four subunits and was inactive. Subunits of the enzyme resulting from dissociation by use of Tris were reconstituted to an active form by placing the preparation in 50 m*M* KCl.

Welch et al. [68] conducted additional studies on the effects of univalent cations on the activity of formyltetrahydrofolate synthetase and observed that a series of univalent cations at 20 °C prevented inactivation and dissociation into subunits by $Tris^+$. The order of effectiveness of cations in preventing inactivation and the order of effectiveness in activating the enzyme were the same, i.e. $NH_4^+ > Rb^+ > K^+ > Cs^+ > Na^+ > Li^+$. A mixture of substrates also was effective in maintaining stability at 20 °C. At 37 °C, however, prevention of inactivation required an activating univalent cation in addition to substrates. Formyltetrahydrofolate synthetase exhibited an absolute requirement for an activating univalent cation at 37 °C, but under conditions in which the enzyme was stable (20 °C in the presence of substrate), NH_4^+ only stimulated activity 2—3-fold. NH_4^+ stimulated activity at 20 °C by reducing the K_m for formate by a factor of 10. From these results it is clear that univalent cations have two functions in formyltetrahydrofolate synthetase systems: (a) they stabilize the active polymeric form of the enzyme, and (b) they facilitate binding of formate to the active form. *Welch et al. [68]* concluded that the polymeric enzyme existed in two forms. The form in the presence of substrate alone exhibited a high K_m for formate and the second form in the presence of NH_4^+ exhibited a low K_m for formate. *Welch et al. [68]* concluded that there was a conformational difference between the two forms sufficient to change the affinity for formate but insufficient to alter the sedimentation coefficient. In an investigation of the properties of K^+ activated D-fructose-1-phosphate kinase from *Aerobacter aerogenes, Sapico* and *Anderson [50]* also concluded that two forms of the enzyme existed and that the active form was favored by K^+.

δ-Aminolevulinic acid dehydratase of *Rhodopseudomonas spheroides*, catalyzes the condensation of two molecules of δ-aminolevulinic acid resulting in the formation of porphobilinogen. The enzyme is maximally active in the presence of K^+, Rb^+, NH_4^+, or Li^+, but exhibits only 50 percent of maximum activity in the presence of Na^+ *(Nandi et al. [31])*. *Nandi* and *Shemin [32]* have shown that, in the presence of K^+, the enzyme existed as an equilibrium mixture of monomers, dimers and trimers, and that the monomer was the smallest species that possessed catalytic activity. In the absence of K^+, or in the presence of Na^+, only the monomer was found. The dimeric form was dissociated into the monomeric form in the absence of K^+ and the monomeric form was further dissociated into two subunits by dialysis, or by treatment with urea. In the presence of K^+ these subunits apparently reassociated and were catalytically active. Activation of δ-aminolevulinic dehydratase by K^+, therefore, is accompanied by an association of the enzyme into the polymeric forms.

It has been reported by *Wilson et al. [71]* that the Schlieren pattern of crystalline pyruvate kinase in the analytical ultracentrifuge showed one homogeneous peak of protein regardless of whether the enzyme was dissolved in K^+, Li^+, or $Tris^+$ solutions. Thus, no tendency for this enzyme to dissociate into subunits was observed in environments of either

activator or non-activator univalent cations. The effects of univalent cations on the association of subunits in the enzymes discussed above, therefore, appear to reflect a mechanism of action of univalent cations that is different from the mechanism with pyruvate kinase.

A role of K^+ in the binding of the coenzyme to the apoenzyme has been observed with some enzyme systems. Propanediol dehydratase from *Aerobacter aerogenes* requires coenzyme B_{12} and K^+ for activity. The holoenzyme was resolved into the apoenzyme and coenzyme B_{12} by gel filtration on Sephadex G-25 in the absence of K^+ *(Toraya et al. [66])*. Incubation of the apoenzyme and coenzyme B_{12} in the presence of K^+ resulted in the reconstitution of the catalytically active holoenzyme. In the presence of substrate and K^+, coenzyme B_{12} was not removed from the holoenzyme, but in the presence of substrate and in the absence of K^+, B_{12} coenzyme was completely removed. These results provide convincing evidence that K^+ plays an essential role in the binding of coenzyme B_{12} to the apoenzyme. Also *Toraya et al. [66]* indicated that NH_4^+ would replace K^+ in this capacity.

Serine dehydratases from *E. coli (Dupourque et al. [9])* and from rat liver *Nishimura* and *Greenberg [34])* require pyridoxal phosphate as a coenzyme and exhibit maximal activities in the presence of K^+. Dialysis of rat liver serine dehydratase in a medium lacking K^+ resulted in the dissociation of the holoenzyme into pyridoxal phosphate and apoenzyme *(Pestana* and *Sols [41])*. Inclusion of K^+ in the dialysis buffer essentially prevented the coenzyme·dissociation. The reconstitution of the holoenzyme from apoenzyme and coenzyme was stimulated about 4-fold by K^+ or NH_4^+, whereas, Na^+ was ineffective. In the presence of K^+ the K_m for the interaction of pyridoxal phosphate with the apoenzyme was reduced from 50 m*M* to 1 m*M (Pestaña [40])* indicating that the presence of K^+ markedly increased the affinity of the serine dehydratase apoenzyme for pyridoxal phosphate.

Happold and *Beechey [15]* also have observed that K^+ or NH_4^+ stabilized *E. coli* tryptophanase, an enzyme that also requires pyridoxal phosphate as a coenzyme. They proposed a scheme in which an equilibrium between the active holoenzyme and an inactive apoenzyme complex was maintained in favor of the active holoenzyme by K^+ or NH_4^+. There is convincing evidence, therefore, that the structural integrity of several enzymes is strikingly influenced by the univalent cation environment.

4.2. *Some kinetic effects of univalent cations*

4.2.1. *Allosteric effects*

Kinetic analyses of enzymic reactions have helped to elucidate the mechanisms involved in the regulation of enzyme activity. Recently, the kinetic approach has revealed that univalent cations behave as allosteric effectors in several enzyme systems.

In a detailed kinetic analysis of the raction catalysed by a $Na^+ + K^+$ activated adenosine triphosphatase [$(Na^+ + K^+)$ ATPase] from brain microsomes, *Robinson [47]* observed that the relationship between the rate of Na^+ and K^+ dependent ATP hydrolysis and the concentration of either Na^+ or K^+ in the medium did not follow *Michaelis-Menten* kinetics, but produced *Lineweaver-Burk* plots that curved upwards and linear Hill plots with slopes greater than one. These results, when interpreted in terms of allosteric models, indicated that both K^+ and Na^+ were acting as cooperative homotropic effectors of the $(Na^+ + K^+)$ ATPase. Furthermore, changes in the concentration of either Na^+ or K^+

altered both V_{max} and the K_m for ATP in a manner indicating that Na^+ and K^+ were also heterotropic effectors of the $(Na^+ + K^+)$ ATPase. Estimates of the energy of activation suggested that increases in salt concentration caused increases in the entropy of activation of the $(Na^+ + K^+)$ATPase. This finding suggested that conformational changes occurred during enzyme activity.

More recently *Robinson* [*49*] has studied the kinetics of the K^+ dependent nitrophenylphosphatase (NPPase) that is associated with the microsomal $(Na^+ + K^+)$ATPase, and which is considered to be an integral part of the overall ATPase reaction. As indicated by a Hill plot of results K^+ behaved as a cooperative homotropic effector of NPPase. Also K^+ behaved as a heterotropic effector toward the substrate, affecting the K_m for nitrophenylphosphate. Furthermore, Robinson presented evidence indicating that Na^+ modified the NPPase activity as a result of interaction between separate Na^+ and K^+ binding sites on the enzyme. Although Robinson's interpretations of the kinetics of the $(Na^+ + K^+)$ATPase reaction have been questioned *(Priestland* and *Whittam* [*43*]*)*, recent evidence *(Tobin et al.* [*65*]*)*, *(Robinson* [*48*]*)*, supports the conclusion that univalent cations serve as allosteric effectors in the $(Na^+ + K^+)$ATPase reaction.

The activating effects of univalent cations on certain pyruvate kinases also are of the allosteric type. Pyruvate kinase from liver *(Jiménez de Asúa et al.* [*21*]*)* and from yeast *(Hunsley* and *Suelter* [*19*]*)* exhibits sigmoidal velocity responses to increasing concentrations of the activating univalent cations, NH_4^+ and K^+. These response curves were converted to normal hyperbolic relationships in the presence of fructose-1,6-diphosphate. In contrast to the above pyruvate kinases, the pyruvate kinases from muscle and higher plants do not exhibit cooperative kinetics.

Adenosine-5-monophosphate deaminase from rabbit muscle *(Smiley* and *Suelter* [*58*]*)* exhibits a sigmoidal velocity response to increasing concentrations of the substrate AMP. In the presence of KCl this response curve was converted to a hyperbolic function. The apparent K_m for AMP was lowered in the presence of KCl, but the V_{max} was not altered. K^+ is not required for catalysis by this enzyme but acts as a heterotropic effector.

δ-Aminolevulinic acid dehydratase of *Rhodopseudomonas spheroides* also displays characteristics of an allosteric enzyme. The substrate has a cooperative homotropic effect that gives rise to a sigmoid response curve. The univalent cations K^+, NH_4^+, Rb^+, or Li^+ when added to reactions containing high substrate concentrations, stimulated the maximum velocity of the reaction by approximately 2-fold and behaved as heterotropic effectors towards the substrate, changing the sigmoidal response curves to hyperbolic functions.

Allosteric models propose that the binding of allosteric effectors to topographically distinct sites on an enzyme influences the enzymic properties of the active site by inducing conformational changes in the enzyme. Although the interpretations of kinetic data are not always unequivocal, it is clear that in certain enzyme systems univalent cations possess the properties of allosteric effectors, and in terms of allosteric processes may be considered to influence enzyme activity by inducing conformational changes in some enzyme molecules.

4.2.2. *Effects of hydrostatic pressure*

Recently *Becker* and *Evans* [*4*] have investigated the univalent cation requirement of highly purified β-galactosidase under different hydrostatic pressures. When lactose, or p-nitrophenyl-β-D-galactopyranoside (PNPG) were used as substrates at atmospheric pres-

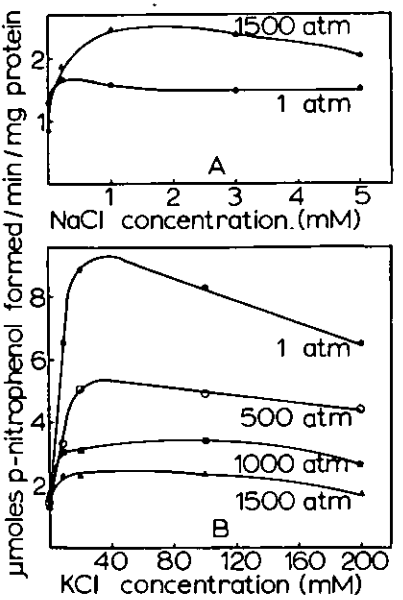

Figure 7. The effects of hydrostatic pressure on the Na^+ (A) and K^+ (B) activated hydrolysis of p-nitrophenyl-β-D-galactopyranoside by β-galactosidase. The incubation mixture (4.0 ml) consisted of 0.1 *M* histidine, 2.3 m*M* p-nitrophenyl-β-D-galactopyranoside, 5 m*M* dithiothreitol and the enzyme preparation (0.72 µg protein). The pressure was varied as indicated. (From *Becker* and *Evans* [4].)

sure, activity was strikingly increased by K^+ but only weakly stimulated by Na^+. Application of hydrostatic pressure ranging up to 1500 atms to β-galactosidase reactions containing o-nitrophenyl-β-D-galactopyranoside (ONPG), PNPG or lactose revealed that increasing pressure increased the rate of hydrolysis when Na^+ was used as the activator, but markedly inhibited reactions with the same three substrates when K^+ was added as the activator. Typical results from these experiments are presented in Figure 7. Control reactions provided convincing evidence that the enzyme was not inactivated by hydrostatic pressure because the hydrolysis in the presence of β-galactosidase continued at a normal rate when hydrostatic pressure was released.

The effects of pressure on the rates of β-galactosidase catalyzed hydrolysis of substrates were interpreted by use of the rationale outlined by *K. J. Laidler* [23]. He has presented an equation from which the volume change, ΔV^*, of the activated enzyme substrate complex may be calculated provided that rates of hydrolysis at different pressures are known. When ONPG and PNPG were utilized as substrates (Table 2) at different pressures in the presence of K^+, ΔV^* was positive. Consequently, K^+ activation apparently occurred with an increase in volume of the activated enzyme-substrate complex. In contrast, in the presence of Na^+ the rates of hydrolysis were increased by pressure and the ΔV^* indicated a decrease in volume of the enzyme-substrate complex during catalysis. Although these experiments indicate effects of univalent cations on volume change of the activated enzyme substrate complex under pressure they do not provide insight into the basis for the differential effects.

Table 2. The effects of hydrostatic pressure on the rate of hydrolysis of o-nitrophenyl-β-D-galactopyranoside and p-nitrophenyl-β-D-galactopyranoside by β-galactosidase*

Substrate	Substrate conc.	Cation	Cation conc.	Pressure	ΔV*
	m*M*		m*M*	atm	cc/mole
o-nitrophenyl-β-D-galactopyranoside	2.3	K^+	10.0	1500	+10.1
	10.0	K^+	30.0	1500	+16.9
	2.3	Na^+	1.0	1500	0.0
	10.0	Na^+	3.3	1500	— 4.2
p-nitrophenyl-β-D-galactopyranoside	2.3	K^+	20.0	1500	+20.4
	10.0	K^+	30.0	500	+ 4.2
	10.0	K^+	30.0	1000	+ 8.6
	10.0	K^+	30.0	1500	+17.9
	2.3	Na^+	1.0	1500	— 6.8
	10.0	Na^+	3.3	1500	— 5.0

* The incubation mixture (4 ml) for the hydrolysis of ONPG and PNPG consisted of the substrate, 5 m*M* dithiothreitol, the appropriate cation chloride addition and the enzyme. The assay mixtures were incubated at 5 °C for 1 hour at the designated pressure. Activity was determined on the basis of the change in absorbance at 410 nm. The volume change (ΔV*) was calculated on the basis of the rates at atmospheric and the applied pressures. (From *Becker* and *Evans [4]*).

In a recent paper *Penniston* [*39*], reported effects of hydrostatic pressure on the activities of a series of enzymes and concluded that the activities of multimeric enzymes were inhibited and the activities of monomeric enzymes stimulated by application of hydrostatic pressure. According to *Penniston*, the dissociation of protein multimers resulting from pressure is a primary cause of decreased enzyme activity. The results of *Becker* and *Evans* [*4*], obtained with multimeric β-galactosidase, show that a change of K^+ to Na^+ in the medium reversed the effects of pressure on enzyme activity. This behavior is not readily explained by an effect of pressure on the dissociation of β-galactosidase into subunits.

In considering the effects of pressure and univalent cations on the activity of β-galactosidase it is useful to review some of the reported effects of pressure on the structure of water in a medium containing univalent cations. *Horne* [*18*] states that highly hydrated cations such as Li^+ and Na^+ contributes toward the association and thus the structure of water molecules, whereas, ions such as K^+ or Rb^+ apparently play no such role. As hydrostatic pressure is applied to hydrated cations in solution, the sphere of water of hydration decreases and thus the local water structure in the immediate vicinity of the cations is destroyed. After the water is stripped away by pressure, the electrical conductivity of solutions containing univalent cations is directly correlated with the crystal ion radii of the cations.

As discussed in a subsequent section of this paper, investigations on the interaction of univalent cations with antibiotic molecules such as nonactin *(Prestegard* and *Chan* [*42*]*)* have revealed that the sphere of water of hydration of univalent cations is lost as the cation is complexed with antibiotic. If one extrapolates results obtained from model systems such as univalent cation-antibiotic complexes and utilizes the reported information on the effects of pressure on the hydration sphere of univalent cations toward an interpretation of the effects of pressure on β-galactosidase, the following arguments would seem rational: (a) at atmospheric pressure, K^+, a weakly hydrated but efficient activator of β-galactosidase might be expected to be bound without its water of hydration to an enzyme site of dimensions appropriate for complexation with K^+; (b) at high hydrostatic pressure one might postulate an alteration in the dimensions of a univalent cation binding site to an

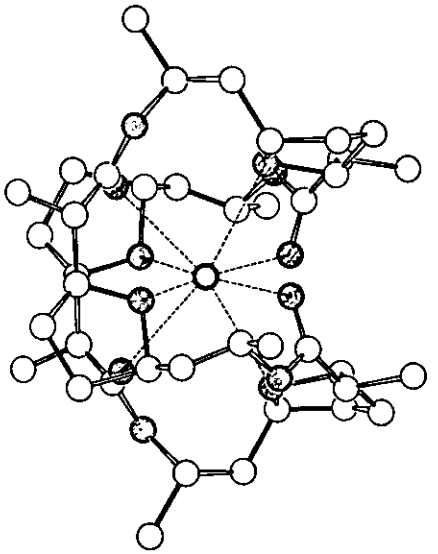

Figure 8. The nonactin-K^+ complex viewed down the *b* crystal axis. Carbon atoms are represented by open circles, oxygen by shaded circles and K^+ by the heavy circle. (From *Kilbourn et al.* [22].)

extent that K^+ no longer could form an appropriate complex and as a consequence enzyme activity might be inhibited; (c) in contrast, when pressure is applied to β-galactosidase in an environment of Na^+ a loss of the hydration sphere of the cation would be expected. The decrease in the hydrated radius of Na^+ resulting from pressure might be expected to allow the Na^+ ion to more closely fit the binding site. Under these conditions, Na^+ dependent catalysis would be increased by pressure. The possibility cannot be ruled out that pressure results in the dissociation of β-galactosidase in a K^+ environment and that dissociation is prevented in a Na^+ environment. It will be of great interest to determine whether improvement of the enzyme activator capacities of Na^+ by pressure is a general phenomenon applicable to whole series of univalent-cation-activated enzymes.

4.3. *Potassium-antibiotic complexes as models*

Investigations of the effects of certain antibiotics on mitochondria have revealed that some of these molecules promote the transport of ions across membranes. Gramicidin and valinomycin both inhibit oxidative phosphorylation by mitochondria and facilitate the transport of external K^+ into the mitochondria. Gramicidin is relatively nonspecific, facilitating the movement of K^+, Li^+, Na^+, Rb^+ and Cs^+, but valinomycin accelerates transport or K^+, Rb^+ or Cs^+, but not Na^+ or Li^+. The macrotetrolide, nonactin, is highly specific for the transfer of K^+ and Rb^+ across membranes but fails to stimulate the transport of Na^+. Detailed chemical investigations of some of these types of antibiotics have provided basic information on the nature of the univalent cation complexation and the mechanisms involved in the transport of ions through membranes. As an example of these investigations, *Kilbourn and associates* [22] have utilized X-ray diffraction to establish the three-dimensional structure of the K^+ complex of nonactin (Figure 8). The

authors state that K^+ is surrounded by 4 oxygen atoms from the furane rings and by 4 oxygen atoms of keto-groups forming an approximate cubic eight coordination. This type of complexation provides a plausible explanation of how nonactin facilitates K^+ transport through membranes.

Prestegard and *Chan* [*42*] have studied the K^+ complex of nonactin by use of NMR and concluded that K^+ was bound to the nonactin molecule without its water of hydration. They observed a change in the conformation of the nonactin ring upon formation of the K^+ complex. In their opinion the nonactin molecule is quite flexible and thus, the size of the apperture in the ring was considered unlikely to be the controlling factor in discriminating between univalent cation species. Since cations must be stripped of their water of hydration before complexation with the antibiotic, they suggest that the hydration energy of the cation may be the controlling factor in cation specificity. This conclusion is consistent with the model of *Diamond* and *Wright* [*8*] which correctly predicts observed patterns of ion specificity by comparing the energy of the ion-site interaction with the free energy of hydration of the univalent cations. The results of *Ovchinnikov et al.* [*38*] concerning the conformation of the K^+ complex of enniatin B are in general agreement with those of *Prestegard* and *Chan* [*42*].

The detailed definitive information that is now available on the univalent cation complexes of antibiotics may serve as useful model information for the interpretation of the role of univalent cations in enzyme catalysis. By extrapolation it would seem logical to assume that univalent cations may lose their water of hydration as they form complexes with univalent-cation-requiring enzymes. Furthermore, it would seem reasonable to conclude that the interaction of univalent cations with enzymes, or enzymes to which substrates are attached, may result in conformation changes in enzyme-substrate-cation complexes. It will be of great interest to learn whether segments of univalent-cation-requiring enzymes may be obtained that possess a capacity to react with univalent cations, forming complexes analogous to the univalent-cation-antibiotic-complexes.

5. *Conclusions*

Potassium is a cofactor for a large number of enzymes that participate in several major metabolic processes. In this review we have considered K^+, and other univalent cations, as necessary cofactors for the syntheses of protein and starch, two metabolic processes of primary economic importance for food production by the agricultural industry. In addition we have summarized information that is considered pertinent to an understanding of the biochemical role of K^+ and other univalent cations in enzymic catalysis.

In the series of steps involved in protein synthesis, K^+ or other univalent cations are rquired for: (a) the synthesis of effective ribosomes; (b) the assembly of functional polyribosomes; (c) the enzymic synthesis of some of but not all of the aminoacyl-tRNA's; (d) the binding of aminoacyl-tRNA's to ribosomes; (e) peptide bond synthesis by the peptidyl transferase reaction, and (f) very likely for the degradation and thus turnover of mRNA during protein synthesis. In regard to the univalent cation specificity for cell-free protein synthesis NH_4^+, K^+ and Rb^+ generally are effective activators for the overall reaction and the individual steps, but neither Na^+ nor Li^+ are effective. In the synthesis of nitrate reductase *in vivo* by *Neurospora crassa* K^+ is the most effective univalent cation, Rb^+ is partially effective but neither Na^+, Li^+ nor NH_4^+ substitute for K^+ in this process.

The failure of NH_4^+ to function in the synthesis of nitrate reductase, however, is a specific case perhaps related to the established capacity of NH_4^+ to function as a repressor of the synthesis of nitrate reductase. A concentration of at least 0.05*M* KCl is necessary for the vigorous activity of the protein synthesizing apparatus in *E. coli (Lubin* and *Ennis* [25]*)*.The concentration of NH_4^+ in normal *E. coli* cells however ranges from 5 to 10 m*M* which is insufficient to satisfy the univalent cation requirements for protein synthesis *(Lubin* and *Ennis* [25]*)*.

Since appreciable concentrations of NH_4^+ are generally toxic to organisms and since only traces of Rb^+ are found in nature, K^+ is the only univalent cation present in living material in sufficient quantity to satisfy the requirements for protein synthesis.

The starch synthetase reaction also requires K^+, NH_4^+ or Rb^+ at relatively high concentrations (i.e. 0.05 *M*), but in this reaction K^+ again is the only univalent cation present in normal tissues in sufficient quantity to meet the requirements for starch synthesis.

In a consideration of the specific mechanisms whereby K^+ or other univalent cations directly or indirectly influence enzyme catalysis the following general conclusions have been made: (a) the association and dissociation of some but not all of the multimeric-univalent-cation-activated enzymes are strikingly influenced by the univalent cation species that predominate in the enzyme environment. Generally, activator cations such as K^+ favor association, whereas nonactivator cations such as $Tris^+$ or Na^+ contribute toward subunit dissociation; (b) another group of univalent-cation-activated enzymes that require a coenzyme such as pyridoxal phosphate or coenzyme B_{12} retain a capacity to effectively bind their particular coenzyme in an activator univalent cation (i.e. K^+) environment, but lose coenzyme binding capacity in an environment where nonactivator cations such as $Tris^+$ or Na^+ predominate; (c) univalent cations function as allosteric effectors for some univalent-cation requiring enzymes suggesting an effect on the interaction of enzyme subunits; (d) in other groups of univalent-cation-activated enzymes, activator univalent cations such as K^+ or Rb^+ induce an active conformation while nonactivator cations such as Na^+ or Li^+ induce an inactive enzyme conformation. Such physical changes may be relatively minor, and may not be detected by ultracentrifugation or electrophoresis methods that ordinarily are used to detect gross physical changes in protein structure.

By extrapolation of information obtained from investigations of the interaction of univalent cations with those antibiotics that are known to facilitate cation transport it is suggested that the hydration sphere of water surrounding univalent cations is lost as the cations bind to enzyme molecules, and that the binding of univalent cations to enzyme molecules may alter their conformation. Furthermore, the evidence indicates that cation specificity for enzyme activation is determined by the energy of ion-site interaction and the free energy of hydration of the univalent cation. Some of the interactions of univalent cations with enzymes may involve a complex of certain enzyme sites and keto-enol reaction intermediates (as suggested by *Suelter)*. It is obvious from this review, however, that univalent cations profoundly influence the properties of many of the univalent-cation-activated enzymes in the absence of reaction intermediates.

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List of abbreviations

AMP	adenosine-5'-monophosphate
ADP	adenosine-5'-diphosphate
ATP	adenosine-5'-triphosphate
ATPase	adenosine-5'-triphosphatase
ADPG	adenosine diphosphate glucose
C.D.	circular dichroism
DNA	deoxyribonucleic acid
GDP	guanosine-5'-diphosphate
GTP	guanosine-5'-triphosphate
GTPase	guanosine-5'-triphosphatase
NMR	nuclear magnetic resonance
NPPase	p-nitrophenyl phosphatase
ONPG	o-nitrophenyl-β-D-galactopyranoside
PNPG	p-nitrophenyl-β-D-galactopyranoside
PEP	phosphoenolpyruvate
Poly U	polyuridylic acid
PRR	proton relaxation rate
RNA	ribonucleic acid
RNase II	ribonuclease II
mRNA	messenger ribonucleic acid
tRNA	transfer ribonucleic acid
rRNA	ribosomal ribonucleic acid
TMA	tetramethylammonium
Tricine	N-tris(hydroxymethyl)methylglycine
Tris	tris (hydroxymethyl)aminomethane
UDPG	uridine diphosphate glucose
ΔV^*	volume change accompanying the transformation of enzyme and substrate into an activated complex

Bibliography

1. *Akatsuka, T.*, and *Nelson, O.E.*: Starch Granule-Bound Adenosine Diphosphate Glucose-Starch Glucosyl Transferase of Maize Seeds. J. Biol. Chem. *241*, pp. 2280–2286 (1966).
2. *Barnard, E.A.*: Ribonucleases. Annu. Rev. Biochem. *38*, pp. 677–732 (1969).
3. *Bayley, S.T.*, and *Kushner, D.J.*: The Ribosomes of the Extremely Halophilic Bacterium, *Halobacterium cutirubrum*. J. Mol. Biol. *9*, pp. 654–669 (1964).
4. *Becker, V.E.*, and *Evans, H.J.*: The Influence of Monovalent Cations and Hydrostatic Pressure on β-Galactosidase Activity. Biochim. Biophys. Acta *191*, pp. 95–104 (1969).
5. *Betts, G.F.*, and *Evans, H.J.*: The Effect of Cations on the Electrophoretic Mobility and Substrate Binding Properties of Pyruvate Kinase. Biochim. Biophys. Acta *167*, pp. 190–193 (1968).
6. *Boulter, D.*: Protein Synthesis in Plants. Annu. Rev. Plant Physiol. *21*, pp. 91–114 (1970).
7. *Conway, T.W.*, and *Lipmann, F.*: Characterization of a Ribosome-Linked Guanosine Triphosphatase in *Escherichia coli* Extracts. Proc. Nat. Acad. Sci. U.S.A. *52*, pp. 1462–1469 (1964).
8. *Diamond, J.M.*, and *Wright, E.M.*: Biological Membranes: The Physical Basis of Ion and Nonelectrolyte Selectivity. Annu. Rev. Physiol. *31*, pp. 582–642 (1969).
9. *Dupourque, D., Newton, W.A.*, and *Snell, E.E.*: Purification and Properties of D-Serine Dehydrase from *Escherichia coli*. J. Biol. Chem. *241*, pp. 1233–1238 (1966).
10. *Ennis, H.L.*: Role of Potassium in the Regulation of Polysome Content and Protein Synthesis in *Escherichia coli*. Arch. Biochem. Biophys. *143*, pp. 190–200 (1971).
11. *Ennis, H.L.*, and *Lubin, M.*: Pre-Ribosomal Particles Formed in Potassium Depleted Cells. Biochim. Biophys. Acta *95*, pp. 605–623 (1965).
12. *Ennis, H.L.*, and *Lubin, M.*: Selective Synthesis of Ribosomal Protein During Recovery from Unbalanced Growth. Biochim. Biophys. Acta *95*, pp. 624–633 (1965).
13. *Evans, H.J.*, and *Sorger, G.*: Role of Mineral Elements with Emphasis on the Univalent Cations. Annu. Rev. Plant Physiol. *17*, pp. 47–76 (1966).
14. *Hall, W.K.*: Potassium and Sodium in Biological Systems. In: The Encyclopedia of Biochem. Eds: Williams, R.J., and Lansford, E.M., pp. 679–682. Reinhold Publishing Co., 1967.
15. *Happold, F.C.*, and *Beechey, R.B.*: Univalent Metals and Other Loose or Non-Specific Activations. Biochem. Soc. Symp. (Cambridge, Engl.) *15*, pp. 52–63 (1958).
16. *Heintz, R.L., Salas, M.L.*, and *Schweet, R.S.*: Phenylalanyl Puromycin Formation as a Model System for Studying Peptide Bond Formation. Arch. Biochem. Biophys. *125*, pp. 488–496 (1968).
17. *Himes, R.H.*, and *Wilder, T.*: Formyltetrahydrofolate Synthetase: Mechanism of Cation Activation. Biochim. Biophys. Acta *99*, pp. 464–475 (1965).
18. *Horne, R.A.*: The Effect of Pressure on the Electrical Conductivity of Aqueous Solutions. Advances in High Pressure Research *2*, pp. 169–223 (1969).
19. *Hunsley, J.R.*, and *Suelter, C.H.*: Yeast Pyruvate Kinase. II. Kinetic Properties. J. Biol. Chem. *244*, pp. 4819–4822 (1969).
20. *Ibuki, F.*, and *Moldave, K.*: Evidence for the Enzymatic Binding of Aminoacyl Transfer Ribonucleic Acid to Rat Liver Ribosomes. J. Biol. Chem. *243*, pp. 791–798 (1968).
21. *Jiménez de Asúa, L., Rozengurt, E.*, and *Carminatti, H.*: Some Kinetic Properties of Liver Pyruvate Kinase (Type L). III. Effect of Monovalent Cations on Its Allosteric Behavior. J. Biol. Chem. *245*, pp. 3901–3905 (1970).
22. *Kilbourn, B.T., Dunitz, J.D., Pioda, L.A.R.*, and *Simon, W.*: Structure of the K^+ Complex with Nonactin, A Macrotetrolide Antibiotic Possessing Highly Specific K^+ Transport Properties. J. Mol. Biol. *30*, pp. 559–563 (1967).
23. *Laidler, K.J.*: The Influence of Pressure on the Rates of Biological Reactions. Arch. Biochem. *30*, pp. 226–236 (1951).
24. *Lubin, M.*: Intracellular Potassium and Control of Protein Synthesis. Federation Proc. *23*, pp. 994–1001 (1964).
25. *Lubin, M.*, and *Ennis, H.L.*: On the Role of Intracellular Potassium in Protein Synthesis. Biochim. Biophys. Acta *80*, pp. 614–631 (1964).
26. *Maden, B.E.H.*, and *Monro, R.E.*: Ribosome-Catalyzed Peptidyl Transfer. Effects of Cations and pH Value. Eur. J. Biochem. *6*, pp. 309–316 (1968).
27. *Mildvan, A.S.*, and *Cohn, M.*: The Interaction of Potassium with Pyruvate Kinase. Abst. Sixth Intern. Congr. Biochem. IUB *32*, p. 322 IV–III (1964).
28. *Mildvan, A.S.* and *Cohn, M.*: Kinetic and Magnetic Resonance Studies of the Pyruvate Kinase Reaction. J. Biol. Chem. *214*, pp. 1178–1193 (1966).
29. *Murata, T.*, and *Akazawa, T.*: Enzymic Mechanism of Starch Synthesis in Sweet Potato Roots. I. Requirement of Potassium Ions for Starch Synthetase. Arch. Biochem. Biophys. *126*, pp. 873–879 (1968).
30. *Murata, T.*, and *Akazawa, T.*: Stimulative Effect of Potassium Ion on Starch Synthetase of Different Plant Origins. Plant Cell. Physiol. *10*, pp. 457–460 (1969).
31. *Nandi, D.L., Baker-Cohen, K.F.*, and *Shemin, D.*: δ-Aminolevulinic Acid Dehydratase of *Rhodopseudomonas spheroides*. I. Isolation and Properties. J. Biol. Chem. *243*, pp. 1224–1230 (1968).
32. *Nandi, D.L.*, and *Shemin, D.*: δ-Aminolevulinic Acid Dehydratase of *Rhodopseudomonas spheroides*. II. Association to Polymers and Dissociation to Subunits. J. Biol. Chem. *243*, pp. 1231–1235 (1968).

33. *Natori, S.*, and *Mizuno, D.*: Turnover of Ribosomal RNA in a Ribonuclease I-Less Mutant of *Escherichia coli*, Q-13, Which Was Found to Possess Polynucleotide Phosphorylase. Biochim. Biophys. Acta *145*, pp. 328–336 (1967).
34. *Nishimura, J.S.*, and *Greenberg, D.M.*: Purification and Properties of L-Threonine Dehydrase of Sheep Liver. J. Biol. Chem. *236*, pp. 2684–2691 (1961).
35. *Nitsos, R.E.*, and *Evans, H.J.*: Effects of Univalent Cations on the Inductive Formation of Nitrate Reductase. Plant Physiol. *41*, pp. 1499–1504 (1966).
36. *Nitsos, R.E.*, and *Evans, H.J.*: Effect of Univalent Cations on the Activity of Particulate Starch Synthetase. Plant Physiol. *44*, pp. 1260–1266 (1969).
37. *Oji, Y.*, and *Izawa, G.*: Effects of Univalent Cations on the Formation of Nitrate Reductase and Nitrite Reductase in Rice Seedlings. Plant Cell Physiol. *10*, pp. 665–674 (1969).
38. *Ovchinnikov, Yu.A., Ivanov, V.T., Evstratov, A.V., Bystrov, V.F., Abdullaev, N.D., Popov, E.M., Lipkind, G.M., Arkhipova, S.F., Efremov, E.S.*, and *Shemyakin, M.M.*: The Physicochemical Basis of the Functioning of Biological Membranes: Dynamic Conformational Properties of Enniatin B and Its K^+ Complex in Solution. Biochem. Biophys. Res. Commun. *37*, pp. 668–676 (1969).
39. *Penniston, J.T.*: High Hydrostatic Pressure and Enzymic Activity: Inhibition of Multimeric Enzymes by Dissociation. Arch. Biochem. Biophys. *142*, pp. 322–332 (1971).
40. *Pestanña, A.*, Opposite Effects of Potassium Ions on the Affinities of Rat Liver Serine Dehydratase for Coenzyme and Substrate. FEBS Letters *13*, pp. 59–61 (1971).
41. *Pestanña, A.*, and *Sols, A.*: Reversible Inactivation of Rat Liver Serine Dehydratase by Its Substrates. FEBS Letters *7*, pp. 29–31 (1970).
42. *Prestegard, J.H.*, and *Chan, S.I.*: Proton Magnetic Resonance Studies of the Cation-Binding Properties of Nonactin. I. The K^+-Nonactin Complex. Biochemistry *8*, pp. 3921–3927 (1969).
43. *Priestland, R.N.*, and *Whittam, R.*: The Influence of External Sodium Ions on the Sodium Pump in Erythrocytes. Biochem. J. *109*, pp. 369–374 (1968).
44. *Ravel, J.M., Shorey, R.L., Froehner, S.*, and *Shive, W.*: A Study of the Enzymic Transfer of Aminoacyl-RNA to *Escherichia coli* Ribosomes. Arch. Biochem. Biophys. *125*, pp. 514–526 (1968).
45. *Ray, R.K.*, and *Burma, D.P.*: Partial Purification and Properties of a K^+- and Mg^{2+}-Dependent Phosphodiesterase from *Salmonella typhimurium*. Biochim. Biophys. Acta *212*, pp. 102–110 (1970).
46. *Reuben, J.*, and *Cohn, M.*: Magnetic Resonance Studies of Manganese (II) Binding Sites of Pyruvate Kinase. Temperature Effects and Frequency Dependence of Proton Relaxation Rates of Water. J. Biol. Chem. *245*, pp. 6539–6546 (1970).
47. *Robinson, J.D.*: Kinetic Studies on a Brain Microsomal Adenosine Triphosphatase. Evidence Suggesting Conformational Changes. Biochemistry *6*, pp. 3250–3258 (1967).
48. *Robinson, J.D.*: Allosteric Interactions with the $(Na^+ + K^+)$-Dependent Adenosine Triphosphatase. Nature *220*, pp. 1325–1326 (1968).
49. *Robinson, J.D.*: Kinetic Studies on a Brain Microsomal Adenosine Triphosphatase. II. Potassium-Dependent Phosphatase Activity. Biochemistry *8*, pp. 3348–3355 (1969).
50. *Sapico, V.*, and *Anderson, R.L.*: Regulation of D-Fructose 1-Phosphate Kinase by Potassium Ion. J. Biol. Chem. *245*, pp. 3252–3256 (1970).
51. *Schneider, Z., Pech, K.*, and *Pawelkiewicz, J.*: Enzymic Transformation of Glycerol to β-Hydroxypropionic Aldehyde. II. Dissociation of the Enzyme into Two-Protein Fragments. Bull. Acad. Polonaise Sci. *14*, pp. 7–12 (1966).
52. *Schweet, R.S., Arlinghaus, R., Shaeffer, J.*, and *Williamson, A.* Studies on Hemoglobin Synthesis. Medicine (Baltimore) *43*, pp. 731–745 (1964).
53. *Schweet, R.S., Holley, R.W.*, and *Allen, G.H.*: Amino Acid Activation in Hog Pancreas. Arch. Biochem. Biophys. *71*, pp. 311–325 (1957).
54. *Scott, J.M.*, and *Rabinowitz, J.C.*: The Association-Dissociation of Formyltetrahydrofolate Synthetase and Its Relation to Monovalent Cation Activation of Catalytic Activity. Biochem. Biophys. Res. Commun. *29*, pp. 418–423 (1967).
55. *Shaeffer, J., Arlinghaus, R.*, and *Schweet, R.*: Effect of Varying the KCl and $MgCl_2$ Concentration on the Enzymic and Non-Enzymic Binding of Phenylalanyl-RNA to Reticulocyte Ribosomes. Arch. Biochem. Biophys. *125*, pp. 614–622 (1968).
56. *Singer, M.F.*, and *Tolbert, G.*: Purification and Properties of a Potassium Activated Phosphodiesterase from *Escherichia coli*. Biochemistry *4*, pp. 1319–1330 (1965).
57. *Skogerson, L.*, and *Moldave, K.*: Evidence for Aminoacyl-tRNA Binding, Peptide Bond Synthesis, and Translocase Activities in the Aminoacyl Transfer Reaction. Arch. Biochem. Biophys. *125*, pp. 497–505 (1968).
58. *Smiley, K.L., Jr.*, and *Suelter, C.H.*: Univalent Cations as Allosteric Activators of Muscle Adenosine 5'-Phosphate Deaminase. J. Biol. Chem. *242*, pp. 1980–1981 (1967).
59. *Sorger, G.J.*: Simultaneous Induction and Repression of Nitrate Reductase and TPNH Cytochrome *c* Reductase in *Neurospora crassa*. Biochim. Biophys. Acta *99*, pp. 234–245 (1965).
60. *Sorger, G.J., Ford, R.E.*, and *Evans, H.J.*: Effect of Univalent Cations on the Immunoelectrophoretic Behavior of Pyruvate Kinase. Proc. Nat. Acad. Sci. U.S.A. *54*, pp. 1614–1621 (1965).
61. *Spahr, P.F.*: Purification and Properties of Ribonuclease II from *Escherichia coli*. J. Biol. Chem. *239*, pp. 3716–3726 (1964).
62. *Spahr, P.F.*, and *Schlessinger, D.*: Breakdown of Messenger Ribonucleic Acid by a Potassium Activated Phosphodiesterase from *Escherichia cali*. J. Biol. Chem. *238*, pp. 2251–2253 (1963).

63. *Spyrides, G.J.:* The Effect of Univalent Cations on the Binding of sRNA to the Template-Ribosome Complex. Proc. Nat. Acad. Sci. U.S.A. *51*, pp. 1220–1226 (1964).
64. *Sueiter, C.H.:* Enzymes Activated by Monovalent Cations. Science *168*, pp. 789–795 (1970).
65. *Tobin, T., Banerjee, S.P.,* and *Sen, A.K.:* Allosteric Interactions in $(Na^+ + K^+)$-ATPase. Nature *225*, pp. 745–746 (1970).
66. *Toraya, T., Sugimoto, Y., Tamao, Y., Shimizu, S.,* and *Fukui, S.:* Potassium Ion as an Essential Factor for Binding of Vitamin B_{12} Coenzyme to Apopropanediol Dehydratase. Biochem. Biophys. Res. Commun. *41*, pp. 1314–1320 (1970).
67. *Waldenström, J.:* Some Properties of Lysyl Ribonucleic Acid Synthetase from *Escherichia coli.* Eur. J. Biochem. *5*, pp. 239–245 (1968).
68. *Welch, W.H., Irwin, C.L.,* and *Himes, R.H.:* Observations on the Monovalent Cation Requirements of Formyltetrahydrofolate Synthetase. Biochem. Biophys. Res. Commun. *30*, pp. 255–261 (1968).
69. *Wildes, R.A., Evans, H.J.,* and *Becker, R.R.:* The Effect of Univalent Cations on the Circular Dichroism of Pyruvate Kinase. Biochim. Biophys. Acta *229*, pp. 850–854 (1971).
70. *Wilson, R.H.* and *Evans, H.J.:* The Effect of Potassium and Other Univalent Cations on the Conformation of Enzymes. In: The Role of Potassium in Agriculture. Eds: Kilmer V.J., Younts, S.W., and Brady, N.C., pp. 189–202, Amer. Society of Agronomy, Madison, Wisconsin 1968.
71. *Wilson, R.H., Evans, H.J.,* and *Becker, R.R.:* The Effect of Univalent Cation Salts on the Stability and on Certain Physical Properties of Pyruvate Kinase. J. Biol. Chem. *242*, pp. 3825–3832 (1967).
72. *Yu, C.T.,* and *Hirsh, D.:* The Stimulatory Effect of Ammonium or Potassium Ions on the Activity of Leucyl-tRNA Synthetase from *Escherichia coli.* Biochim. Biophys. Acta *142*, pp. 149–154 (1967).

The Effect of Potassium Deficiency on the Fine Structure of Proplastids

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Summary

Electronmicroscopic investigations were carried out with corn root tips after removal of potassium. Potassium was removed by treatment with 25 mM NaCl solution or by treatment with the antibiotic mycostatin. After these treatments the potassium content of the corn root tips decreased to 35–15% of the initial content. The removal of potassium caused a remarkable change in the fine structure of the proplastids. The proplastids were swollen, the matrix appeared thin and had many electrontransparent areas. This appearance did not occur in treatments with 25 mM KCl or in treatments where besides mycostatin 10 mM KCl were present. Therefore these changes in the fine structure of proplastids are obviously specific symptoms of potassium deficiency. The findings are discussed in relation to the functions of potassium in carbohydrate and protein metabolism.

1. *Introduction*

Known visual symptoms of potassium deficiency in higher plants are brown necrotic spots on the older leaves and stems. In his anatomical studies of deficiency symptoms using the light microscope *Bussler* [2] observed that the development of these necrotic spots begins with a collapse of the cells in the outer cell layers. Electronmicroscopic investigations on plants with potassium deficiency were carried out by several authors, who found a destruction of the chloroplast structure *(Thomson* and *Weier* [*14*], *Vesk et al.* [*15*]*)* and a breakdown of mitochondria *(Kursanov* and *Vyskrebentzeva* [*6*]*)*. But it is questionable if changes in the fine structure observed in these long-term studies are typical and specific symptoms of potassium deficiency. Plants growing without potassium for a long time may not only show potassium deficiency but also unspecific symptoms. For instance the disturbance of the carbohydrate metabolism by potassium deficiency may cause retardation of growth and symptoms of senescence.

In order to avoid such unspecific symptoms we used methods by which potassium can be removed in a very short time. For this purpose we used root tips of corn *(Zea mays)* in which potassium was removed by exchange for sodium (treatment with sodium chloride) or by treatment with the antibiotic mycostatin which increases the permeability of the cell membranes specifically for potassium.

2. *Experimental*

Root tips of Zea mays were treated for several hours in the following solutions (24 °C):

1. buffer* alone

* 0.8 meq NaH_2PO_4, pH 5.5

2. buffered 25 m*M* NaCl solution
3. buffered 25 m*M* KCl solution
4. buffered 4×10^{-5} *M*** mycostatin solution
5. buffered 4×10^{-5} *M* mycostatin solution + 10 m*M* KCl

Potassium and sodium were determined by flame photometry.

The method for the *electronmicroscopic investigations* was as follows: Fixation: glutaraldehyde/OsO_4; embedding: epon-araldit: ultra thin sections: Ultrotome III, *LKB* Stockholm; contrast: uranyl acetate, lead citrate; photographs: electronmicroscope EM 9, Fa. Zeiss.

3. *Results*

3.1 *Potassium content and changes in fine structure of corn root tips*

The influence of the different treatments on potassium content and fine structure of the proplastids is shown in Table 1. The potassium content of the untreated root tips was 75 eq/g freshweight. After treatment with phosphate buffer alone there was still 89% of the initial potassium content present. After treatment with 25 m*M* NaCl for 8 hours the potassium content decreased to 15%, after treatment with mycostatin for 2 hours to 37% and after treatment with mycostatin for 4 hours to 17% of the initial content.

In both cases, after removal of potassium by treatment with NaCl or by treatment with mycostatin, remarkable changes occured in the fine structure of the proplastids. In the controls (untreated, buffer alone) the proplastids had a dense matrix and well defined inner membranes (Figure 2), but after removal of potassium the proplastids were swollen, the matrix of proplastids appeared thin and there were many electrontransparent areas (Figures 1, 3). This swelling of proplastids was not caused by an unspecific salt effect, because it did not occur, when corn root tips were treated with 25 m*M* KCl (Figure 2) instead of NaCl. After treatment with 25 m*M* KCl the matrix of the proplastids was not swollen, but was dense and did not differ from the controls (untreated, buffer alone). When besides mycostatin 10 m*M* KCl was also present the root tips still contained 75% of the initial potassium content and changes of the fine structure of the proplastids did not occur. The matrix remained dense as in the controls. Even after treatment with mycostatin for 4

Table 1. Fine structure of proplastids and potassium content of corn root tips after treatment with NaCl or mycostatin (4×10^{-5} m)

Treatment	K % of initial content	proplastids
untreated	100/75/μeq/g FG	dense
8 h buffer	87.8	dense
8 h 25 m*M* NaCl	14.6	*swollen*
8 h 25 m*M* KCl	156.1	dense
2 h mycostatin	36.6	*swollen*
2 h mycostatin + 10 m*M* KCl	78.3	dense
4 h mycostatin	17.0	*swollen*
4 h mycostatin + 10 m*M* KCl	75.0	dense

** Mycostatin (=Nystatin, MG ~ 937, Fa. *Serva*, Heidelberg) was dissolved in 2 ml dimethylformamid and diluted with aqua dest. 1:500.

2. buffered 25 m*M* NaCl solution
3. buffered 25 m*M* KCl solution
4. buffered 4×10^{-5} *M*** mycostatin solution
5. buffered 4×10^{-5} *M* mycostatin solution + 10 m*M* KCl

Potassium and sodium were determined by flame photometry.

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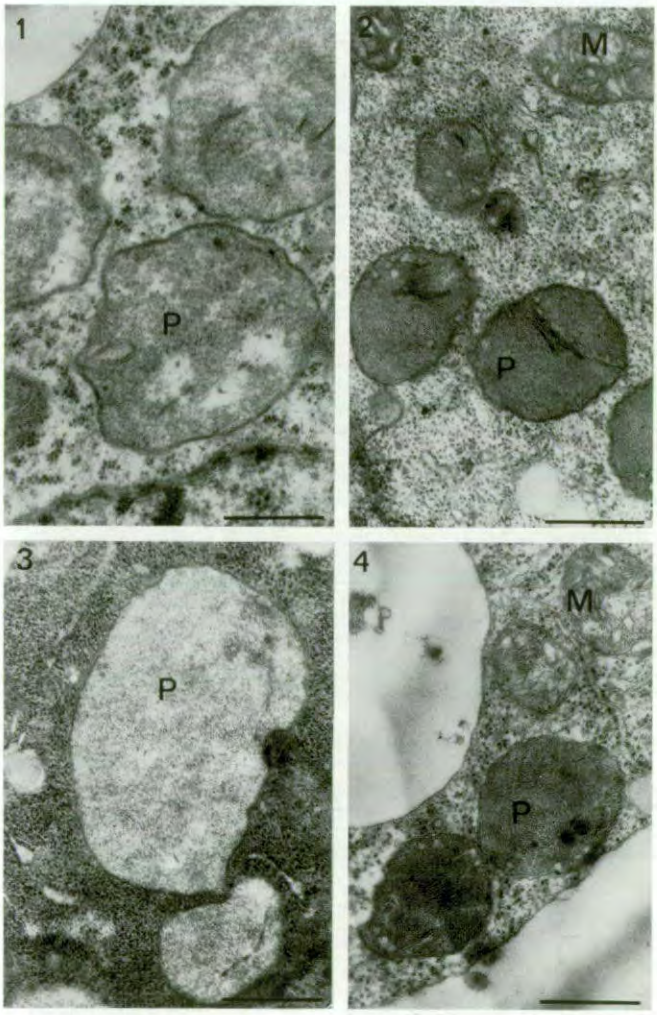

Figure 1. 8 h 25 m*M* NaCl (15% K of the control): The proplastids are swollen. The matrix appears thin and has electron-transparent areas. The ribosomes are aggregated.

Figure 2. 8 h 25 m*M* KCl (156% K of the control): The matrix of the proplastids appears dense. The fine structure of the cells of this treatment was the same as in untreated controls and after treatment with phosphate buffer alone. Therefore Fig. 2 is representative also for both of these treatments.

Figure 3. 4 h mycostatin (17% K of the control): The proplastids are swollen. Similar to the treatment 25 m*M* NaCl (Figure 1) the matrix appears thin and has electron-transparent areas.

Figure 4. 4 h mycostatin – 10 m*M* KCl (75% K of the control): The effect of mycostatin is prevented by the addition of KCl. The proplastids have a dense matrix.

Figure 1–4. Parts of cells of corn root tips. Fixation: glutaraldehyde-OsO_4; micrographs: electronmicroscope EM 9 Zeiss, 18,000:1. The scale marker in all micrographs is equal to 0,5 μm. P = proplastids, M = mitochondria.

hours the proplastids were not altered, provided 10 m KCl was additionally present (Figure 4), but were extremely swollen after 4 hours treatment with mycostatin without KCl (Figure 3).

4. *Discussion*

It was the aim of this investigation to find specific changes in the fine structure of the cell after removal of potassium. The problem was to find a method of removing potassium without causing secondary effects. Our first attempt of inducing deficiency symptoms by treatment with tetraphenylboron, a precipitant for potassium, which removes potassium very quickly, failed, because tetraphenylboron itself causes severe damage of the fine structure and alters the membranes in a remarkable way as has been described previously *(Hecht-Buchholz* and *Marschner [3])*. After treatment with NaCl there occured besides swelling of proplastids several changes in the fine structure: appearance of polysomes instead of monosomes (Figure 1), aggregation of endoplasmic reticulum, accumulation of mitochondria and cytosomes as have been described previously *(Hecht-Buchholz* and *Marschner [4]*, *Hecht-Buchholz et al. [5])*. These changes appeared only after NaCl treatment, but not after treatment with mycostatin, therefore they are not due to the removal of potassium but seem to be specifically caused by sodium. In contrast to this a swelling of proplastids occured in both cases of removal of potassium, i.e. after treatment with NaCl and after treatment with mycostatin. Swelling of proplastids after treatment with mycostatin was prevented when potassium was present simultaneously. Therefore we can consider this swelling of the proplastids as a specific symptom of potassium deficiency. This finding that the effect of mycostatin on the fine structure is overcome by addition of KCl agrees with the findings of *Marini et al. [9]*, who found that the toxic effect of mycostatin on the growth of cells of microorganisms could be eliminated by addition of KCl.

The chloroplasts are known to have a relatively high potassium content *(Larkum [7])*. Consequently a profound effect of potassium deficiency on proplastids is not astonishing. But what are the reasons for swelling of the proplastids after removal of potassium? Although less information is available about the role of proplastids, the fact that starch is built up in proplastids permits the deduction that they play a role in carbohydrate metabolism. Known symptoms of potassium deficiency are decreased activity of starch synthetase *(Nitsos* and *Evans [11])* and increased activity of hydrolytic enzymes such as amylase and saccharase *(Amberger [1]*, *Moll [10])*. This leads to accumulation of soluble carbohydrates, especially monosaccharides and these probably increase the osmotic pressure in the proplastids causing the swelling. Because potassium is obligatory for the synthesis of polypeptides *(Lubin* and *Ennis [8]*, *Spyrides [13])*, the synthesis of proteins is restricted under potassium deficiency conditions. The resulting accumulation of soluble nitrogenous compounds may also cause an increase of osmotic pressure and swelling of the proplastids. Besides swelling of proplastids due to accumulation of soluble compounds as a result of the disturbed carbohydrate and nitrogen metabolism in potassium deficient plants, we may assume an alteration of the protein structure of the proplastids by replacement of potassium by other cations like sodium or hydrogen. In addition to our electronmicroscopic studies we are now carrying out biochemical investigations concerning the carbohydrate and nitrogen compounds under conditions of potassium deficiency. Since for a succesful diagnosis of reasons the employment of different methods is

often indispensable, we do hope that our investigations employing both – electronmicroscopic and biochemical methods-will yield more information about the role of potassium in plant metabolism.

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Bibliography

1. *Amberger, A.:* Einfluß von K und N auf Ferment- und Kohlenhydrathaushalt von Grünlandpflanzen. Z. Pflanzenernähr., Düng., Bodenkd. *66*, 211–222 (1954).
2. *Bussler, W.:* Vergleichende Untersuchungen an Kali-Mangelpflanzen. Verlag Chemie, Weinheim/Bergstraße, 1962.
3. *Hecht-Buchholz, Ch.*, and *Marschner, H.:* Einfluß von Tetraphenylborat auf die Feinstruktur von Zellen der Maiswurzelspitze. Z. Naturforsch. *25 b*, 1477–1479 (1970).
4. *Hecht-Buchholz, Ch.*, and *Marschner, H.:* Veränderungen der Feinstruktur von Zellen der Maiswurzelspitze bei Entzug von Kalium. Z. Pflanzenphysiol. *63*, 416–427 (1970).
5. *Hecht-Buchholz, Ch., Pflüger, R.*, and *Marschner, H.:* Einfluß von Natriumchlorid auf Mitochondrienzahl und Atmung von Maiswurzelspitzen. Z. Pflanzenphysiol., 65, 410–417 (1971).
6. *Kursanov, A.L.*, and *Vyskrebentzeva, E.J.:* Stoffwechsel der Pflanze unter den Bedingungen von Kaliummangel. Agrochimija (Moskau) *11*, 65–77 (1967).
7. *Larkum, A.W.D.:* Ionic relations of chloroplasts in vivo. Nature *218*, 447–449 (1968).
8. *Lubin, M.*, and *Ennis, H.L.:* On the role of intracellular potassium in protein synthesis. Biochim. Biophys. Acta *82*, 614–631 (1964).
9. *Marini, F., Arnow, P.*, and *Lampen, J.O.:* The effect of monovalent cations on the inhibition of yeast metabolism by nystatin. J. gen. Microbiol. *24*, 51–62 (1961).
10. *Moll, A.:* Der Einfluß der N-P-K-Düngung und der Bodenfeuchtigkeit auf den Zuckergehalt von Kartoffelknollen. Z. Pflanzenernähr. u. Bodenkunde *118*, 35–43 (1967).
11. *Nitsos, R.E.*, and *Evans, H.J.:* Effects of univalent cations on the activity of particulate starch synthetase. Plant Physiol. *44*, 1260–1266 (1969).
12. *Sinclair, C.:* Relation between mineral deficiency and amine synthesis in barley. Nature *213*, 214–215 (1967).
13. *Spyrides, G.L.:* The effect of univalent cations on the binding of sRNA to the template-ribosome complex. Proc. Nat. Acad. Sci. *51*, 1220–1226 (1964).
14. *Thomson, W.W.*, and *Weier, T.E.:* The fine structure of chloroplasts from mineral deficient leaves of *Phaseolus vulgaris*. Amer. J. Bot. *49*, 1047–1055 (1962).
15. *Vesk, M., Possingham, V., Mercer, F.V.:* The effect of mineral nutrient deficiencies on the structure of the leaf cells of tomato, spinach and maize. Aust. J. of Bot. *14*, 1–18 (1966).

Effects of Potassium and Sodium on the Contents of Soluble Carbohydrates and Nitrogenous Compounds in Grass

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Summary

Italian ryegrass, grown in pots containing potassium deficient soil with two nitrogen levels and with or without potassium and sodium sulphates applied at equivalent rates, was analysed to assess the effects of replacing potassium by sodium on the organic constituents.
With 40 ppm of nitrogen, potassium but not sodium increased the total yield of two cuts. With 160 ppm of nitrogen, both potassium and sodium increased yields but potassium more so than sodium.
At the lower rate of nitrogen, potassium and sodium decreased the reducing sugars, increased the sucrose but had little effect on the fructosan in the grass of the second cut. With 160 ppm of nitrogen, all the sugars were increased by potassium and sodium, especially fructosan; potassium increased fructosan more than sodium.
Potassium, and to a lesser extent sodium, decreased the free amino acids, ammonium- and nitrate-nitrogen and increased protein nitrogen in the grass given 160 ppm of nitrogen.

1. *Introduction*

There is much evidence that sodium can to some extent replace potassium in its effects on growth of plants [*2,6*] but little work has been done to investigate the effect of this replacement on the organic constituents of the dry matter. Since potassium deficiency in plants has been linked with both carbohydrate and nitrogen metabolism [*2*], work has recently been started at Rothamsted to study how the replacement of potassium by sodium affects various carbohydrate and nitrogen fractions in grass. This paper describes some preliminary results obtained from a pot experiment done in 1969.

2. *Experimental*

The soil used in the experiment was Woburn sandy loam derived from the Lower Greensand. The air-dry soil contained 40 ppm exchangeable K, 13 ppm exchangeable Na and had a pH of 6.5 in water. The experiment tested all combinations of 0 and 160 ppm N (as ammonium nitrate), 0 and 120 ppm K (as potassium sulphate) and 0 and 70 ppm Na (as sodium sulphate). Sodium and potassium were given on an equivalent basis. Each pot containing 2350 g of air-dry soil had a basal dressing of 1.0 g Ca $(H_2PO_4)_2.H_2O$ and 0.3 g $MgSO_4.7H_2O$. On the 13th June Italian ryegrass *(Lolium multiflorum)* S22 was sown. The grass was cut on the 8th July and on the 28th July at 2.0 cm above the soil surface. After the first cut all N and Mg dressings were repeated. The freeze-dried grass from the second cut from all treatments was analysed for soluble carbohydrates but only the grass given the larger N dressing was analysed for nitrogen compounds.

3. *Analytical methods*

Soluble carbohydrates were extracted from plant material with 80% (v/v) ethanol as described by *Nowakowski* [*13*]. Reducing sugars were determined in both the alcohol extract and in a water extract of the residue, both before and after hydrolysis with 0.1 *N* H_2SO_4 using the colorimetric method of *Nelson* [*11*] as modified by *Somogyi* [*15*].
Non-protein nitrogen was extracted from plant material with 80% (v/v) cold ethanol. The ethanol extract was evaporated to dryness under reduced pressure on a rotary evaporator (below 40 °C) and the residue was re-extracted with water (< 40 °C) and made up to a known volume. Various nitrogenous compounds were determined in this solution as described by *Nowakowski et al.* [*14*]. Free amino acids were determined in a portion of the aqueous solution of the ethanol extract, shaken in a separating funnel with three times its volume of chloroform to remove pigments [*1*]. The chloroform-extracted solution was evaporated to dryness under reduced pressure below 40 °C and redissolved in 10 ml 0.01 *N* HCl containing 0.1 μ·mole norleucine. A known volume (0.5–1.5 ml) of this solution was analysed for the individual amino acids using the Technicon Amino Acid Analyser.

4. *Results*

Total dry matter yields of two cuts (Table 1)
With the smaller amount of nitrogen (40 ppm), sodium did not affect yields but potassium did. With the larger amount of nitrogen (160 ppm), both sodium and potassium increased yields; the effect of potassium was significantly greater than sodium. Although there was a negative interaction between potassium and sodium, sodium increased yields significantly even when potassium was given.

Table 1. Effects of nitrogen, potassium and sodium on total yields of two cuts of ryegrass

	g D.M./POT			
	40 ppm N		160 ppm N	
	Without Na	With 70 ppm Na	Without Na	With 70 ppm Na
Without K	7.22	7.37	11.93	15.49
With 120 ppm K	8.01	7.95	16.96	18.38

Standard error ± 0.160

Soluble carbohydrates in the second cut (Table 2)
At the lower rate of nitrogen (40 ppm) potassium and sodium decreased the reducing sugars, increased the sucrose but had little effect on the fructosan content of ryegrass. With the larger dressing of nitrogen (160 ppm), both sodium and potassium increased the content of all sugars, especially fructosan; potassium increased the fructosan content more than sodium. Potassium and sodium given together produced more fructosan than potassium alone.
Nitrogenous compounds in the second cut (Table 3)
In the grass given the larger dressing of nitrogen (160 ppm) both potassium and sodium decreased all the free amino acids, ammonium- and nitrate-nitrogen concentrations, but the effects of potassium were larger than sodium. Dicarboxylic acids and their amides,

Table 2. Effects of nitrogen, potassium and sodium on yields and percentage of soluble carbohydrates in the second cut of ryegrass

Fertilizer supplying (as ppm of weight of soil used)			D.M. yield g/pot	% in Dry Matter			
				Reducing sugars	Sucrose	Fructosan	Total soluble carbohydrates
N	K	Na					
40	0	0	3.94	3.5	2.8	18.8	25.1
40	120	0	4.37	2.7	3.3	19.2	25.2
40	0	70	3.85	2.6	3.7	19.2	25.5
40	120	70	4.23	2.2	4.4	19.6	26.2
160	0	0	6.19	5.7	2.5	0.5	8.7
160	120	0	9.92	8.5	4.9	7.1	20.5
160	0	70	8.80	8.4	4.9	3.5	16.8
160	120	70	10.84	6.7	4.7	14.3	25.7
Standard error			±0.180	±0.16*	±0.13*	±0.48*	—

* Standard error of duplicate estimations on composite samples of three replicates

Table 3. Effects of potassium and sodium on free amino acids, ammonia and nitrate concentrations in the second cut of ryegrass given 160 ppm N

Fertilizer supplying (as ppm of weight of soil used)				
Na	0	0	70	70
K	0	120	0	120
	μg amino acid per 1.0 g dry leaf			
Aspartic acid	549	293	375	396
Asparagine	4050	283	1131	189
Threonine	903	280	360	211
Serine	1304	471	574	334
Glutamic acid	592	225	252	327
Glutamine	6362	730	1252	834
Proline	369	119	134	90
Glycine	129	53	61	44
Alanine	881	732	756	612
Valine	783	237	298	172
Methionine	—	25	—	8
iso-Leucine	369	132	164	99
Leucine	337	183	212	146
Tyrosine	242	119	148	99
Phenylalanine	485	208	258	178
β-Alanine	48	21	tr.	tr.
4-amino-*n*-butyric acid	1265	1024	1119	923
Ethanolamine	336	248	253	182
Lysine	548	169	205	118
Histidine	158	61	29	37
Arginine	318	108	133	78
Ammonia (as NH_4)	990	244	270	103
Nitrate (as NO_3)	6330	381	885	355
Protein N as % of total N	77.9	88.1	87.7	90.7

serine, threonine, valine and lysine were most affected, alanine and 4-amino-*n*-butyric acid least. Free methionine occurred only in grass given potassium. Protein nitrogen was increased by either potassium or sodium and more by both. This result for protein nitrogen resembles that of *Joham* and *Amin* [9] obtained with cotton.

5. *Discussion*

There is little information available on the specific role which sodium plays in the nutrition of plants. This is not surprising since the function of potassium in plants is still a subject for much speculation. The results of this experiment show that the effects of sodium and potassium on both the yields and the organic composition of grass are closely associated and confirm previous findings that whatever physiological processes are disturbed by potassium deficiency, these can be, in part at least, corrected by sodium [*5, 8, 16*].

Grass given sodium as well as potassium yielded more, contained more fructosan and less soluble nitrogen, than grass given potassium alone. This suggests that sodium may have some independent function other than that of replacing potassium in the growth and metabolism of ryegrass. *Lehr* [*10*] discussing the results of twelve experiments with oats gave a similar explanation for the effect of sodium on yields. Although there is no convincing evidence that sodium is an essential nutrient for any agricultural crop, except the halophyte *Atriplex vesicaria* [*4*], sugar beets and mangolds give higher yields when they are given sodium in addition to adequate potassium [*6, 16*]. *Cooke* [*6*] stated that in such crops sodium is able to perform certain functions of potassium, and to perform them better. The hypothesis that sodium may have some independent function in plant metabolism is supported to some extent by *Evans* and *Sorger's* [*7*] views on the mechanism of action of univalent cations on various enzyme systems. They stated that 'those enzymes that are activated by K^+... are activated little by Na^+... Conversely, those few enzymes that are activated by Na^+... are activated much less or not at all by K^+...'. *Bollard* and *Butler* [*3*] suggested that sodium is a 'functional nutrient' (a term proposed by *Nicholas* [*12*]), i.e. a mineral element that may function in some precise way in plant metabolism irrespective of whether or not its action is completely specific or indispensable.

The results of this experiment indicate also that investigations on the effect of K-deficiency in soil on the growth and organic composition of plants must take into account the level of exchangeable sodium in the soil.

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Bibliography

1. *Avapara, J.:* Application of paper chromatography to the estimation of free amino acids in tissues. Arch. Biochem. *19*, 172–173 (1948).
2. *Black, C.A.:* Soil-Plant Relationships 2nd Edn., pp. 731–736. John Wiley & Sons Inc., New York 1967.
3. *Bollard, E.G.*, and *Butler, G.W.:* Mineral nutrition of plants. A. Rev. Pl. Physiol. *17*, 77–112 (1966).
4. *Brownell, P.F.:* Sodium as an essential micronutrient for a higher plant. *(Atriplex vesicaria).* Pl. Physiol., Lancaster *40*, 460–468 (1965).
5. *Coleman, R.G.*, and *Richards, F.J.:* Some aspects of nitrogen metabolism in barley and other plants in relation to potassium deficiency. Ann. Bot. *20*, 393–409 (1956).
6. *Cooke, G.W.:* The Control of Soil Fertility, p. 75. Crosby Lockwood & Son Ltd., London 1967.
7. *Evans, H.J.*, and *Sorger, G.J.:* Role of mineral elements with emphasis on the univalent cations. A. Rev. Pl. Physiol. *17*, 47–76 (1966).
8. *Hylton, L.O., Ulrich, A.*, and *Cornelius, D.R.:* Potassium and sodium interrelations in growth and mineral content of Italian ryegrass. Agron. J. *59*, 311–314 (1967).
9. *Joham, H.E.*, and *Amin, J.V.:* Role of sodium in the potassium nutrition of cotton. Soil Sci. *99*, 220–226 (1965).
10. *Lehr, J.J.:* Sodium as a plant nutrient. J. Sci. Fd. Agric. *4*, 460–471 (1953).
11. *Nelson, N.:* A photometric adaptation of the Somogyi method for the determination of glucose. J. biol. Chem. *153*, 375–380 (1944).
12. *Nicholas, D.J.D.:* Minor mineral nutrients. A. Rev. Pl. Physiol. *12*, 63–90 (1961).
13. *Nowakowski, T.Z.:* Effects of nitrogen fertilizers on total nitrogen, soluble nitrogen and soluble carbohydrate contents of grass. J. agric. Sci., Camb. *59*, 387–392 (1962).
14. *Nowakowski, T.Z., Cunningham, R.K.*, and *Nielsen, K.F.:* Nitrogen fractions and soluble carbohydrates in Italian ryegrass. J. Sci. Fd. Agric. *16*, 124–134 (1965).
15. *Somogyi, M.:* Notes on sugar determination. J. biol. Chem. *195*, 19–23 (1952).
16. *Truog, E., Berger, K.C.*, and *Attoe, O.J.:* Response of nine economic plants to fertilization with sodium. Soil Sci. *76*, 41–50 (1953).

Why Can Sodium Replace Potassium in Plants?

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Summary

The extent of replacement of potassium by sodium depends decidedly upon the plant species. There is a clear positive relationship between the uptake and translocation of sodium to the shoot and the extent of replacement of potassium in the plant species.
In its functions within the vacuole (osmotic pressure) potassium is replaceable to a large extent by sodium because this function is nonspecific. This replacement within the vacuole makes potassium available for specific functions within the cell or for retranslocation.
Compared with potassium the activating effect of sodium on enzymes is generally small. At least in some plant species however sodium also is able to activate enzyme systems remarkably: in these cases the ratio potassium/sodium is of special importance.
From the high concentration of sodium in the chloroplasts of natrophilic plant species one can assume that in these cases sodium can replace potassium at least partially in its function in activating enzymes and in flux processes through thylacoid membranes (photophosphorylation). On the other hand in natrophobic plant species (e.g. beans) an exchange of potassium by sodium in the chloroplasts causes serious changes in the fine structure of these organelles.
In certain plant species like sugar beet sodium shows a high mobility in the phloem; therefore in this species sodium should be able to replace potassium also in its function in long distance transport processes.
In addition to its ability to replace potassium more or less in plant species like sugar beet sodium has also positive effects on growth and composition of these plants. This can be explained with the existence of isoenzymes which show their highest activity when potassium and sodium are simultaneously present.
Sodium can however be clearly shown as essential plant nutrient only in the case of halophytes.

For answering the question why can sodium replace potassium in plants it is necessary first to specify possibility and extent of replacement of potassium by sodium. About this problem many papers are already published (see reviews of *Wybenga* [*82*]; *Baumeister* [*4*]). These are often contradictory however; one reason for this is that the results are obtained mainly from field and pot experiments using soils where – due to the native sodium content of these soils – the sodium content of the plants is already high without sodium fertilization (e.g. *Dorph-Peterson* and *Steenbjerg*, [*16*]). In certain plant species like *Beta vulgaris* however a partial replacement of potassium by sodium can be demonstrated practically in all experiments. This was shown already by *Stoklasa* [*73*] in pot experiments where – besides the replacement of a high proportion of potassium – there was also an additional stimulating effect of sodium on the growth of this plant species. This specific effect of sodium in *Beta vulgaris* could be demonstrated later very often *(Scharrer* and *Kühn* [*65*], *Scharrer et al.* [*66*]*)*, and was also confirmed recently *(Tinker* [*75*]*; Draycott et al.* [*17*]*; El-Sheikh et al.* [*19*]*)*. This question of a specific function of sodium will be discussed later.
With respect to the replacement of potassium by sodium the experiments of *Harmer* and *Benne* [*24*] and *Harmer et al.* [*25*] give the best survey. In the field experiments performed

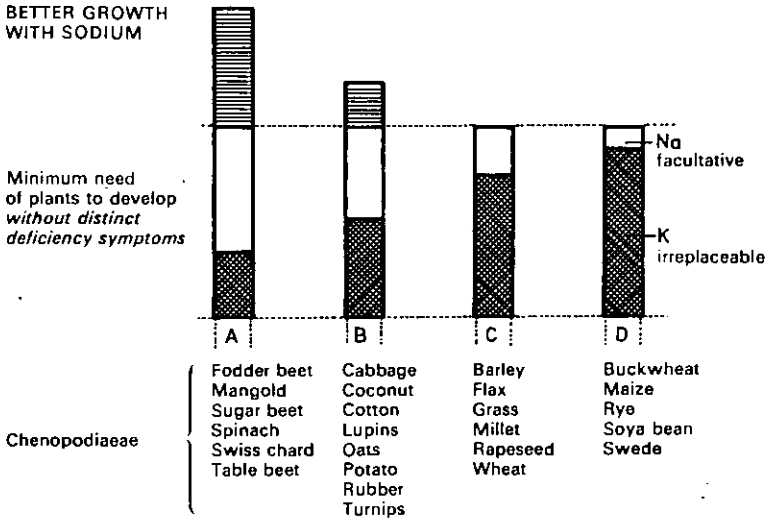

Figure 1. Tentative scheme for classification of crops according to potassium-replacing power of sodium and independent sodium-effect on yield. (From *Lehr*, [*40*]).

by these authors it was shown very clearly that mainly the plant species decides the degree of replacement of potassium by sodium. The results of *Harmer* and *Benne* [*24*] and others were summarized schematically by *Lehr* [*40*] in Figure 1. Certainly one can doubt the absolute proportions in this figure but the tendency is reflected correctly. This is also shown in water culture experiments with several plant species *(Truog et al.* [*76*]; *Montasier et al.* [*49*]*)*. The great differences between the plant species with respect to replacement of potassium by sodium and the specific effect of sodium in certain plant species are generally accepted today and are the basis for answering the question why sodium can replace potassium.

When comparing plant species it is obvious that there is a high variation in the relation potassium/sodium within the plants. In field experiments *(Harmer* and *Benne* [*24*]*)* this relation varies between 0.78 (celery) and 13.39 (asparagus) and is mainly or entirely caused by differences in the sodium content.

In water culture experiments with equivalent supply of potassium and sodium using several plant species *Collander* [*12*] found high variation in the potassium/sodium ratios in the shoots between 0.43 *(Atriplex hortense)* and 44.3 *(Zea mays)*. A comparison of the potassium/sodium ratio in the shoots and effect of sodium especially with respect to its ability to replace potassium clearly shows that if potassium and sodium are supplied in comparable amounts the more the sodium taken up and translocated to the shoot the higher is the replacement *(Bower* and *Wadleigh* [*7*]; *Larson* and *Pierre* [*37*]; *Saalbach* and *Aigner* [*64*]*)*. An example for this is demonstrated for maize and sugar beet in Table 1.

Differences between both plant species are existing already during accumulation in the roots, the differences however are more pronounced during translocation to the shoots. While in maize the sodium taken up is localized mainly in the roots and translocation to the shoots takes place only at higher external concentrations (or in long term experi-

Table 1. Uptake and translocation of potassium and sodium in maize and sugar beet plants (μeq/fresh matter/4 hours) *Marschner* and *Schafarczyk [42].*

External solution meq/l Presence of 1meq Ca^{2+}/l	Maize				Sugar beet			
	Roots		Shoots		Roots		Shoots	
	K	Na	K	Na	K	Na	K	Na
1 NaCl + 1 KCl	9.4	3.3	0.9	0.0	7.5	9.0	2.5	4.5
10 NaCl + 10 KCl	15.0	5.9	3.0	1.4	10.7	15.4	6.1	10.6

ments) in sugar beet sodium is readily translocated into the shoot. Similar conditions as in maize are existing in beans where a preferential accumulation of sodium in the stele of the roots and the stems could be demonstrated *(Wallace et al. [80]; Jacoby [31]; Pearson [55]; Rains [59]).* This preferential accumulation of sodium in the stele takes place also in maize roots *(Shone et al.[68]).* Even during passage through the xylem vessels of the roots there is a preferential accumulation of sodium by the surrounding tissue of the stele; therefore with increasing length of the passage through the roots (Table 2) the relation sodium/potassium in the xylem sap shifts in favour of potassium (from 0.26 to 0.03). One can consider this as a mechanism of regulation that restricts the translocation of sodium to the shoots. In such plant species sodium can replace potassium only to a limited extent.

Table 2. Absorption and translocation of labelled sodium and potassium by detached maize roots of different lengths: Volume of the exudate and Na/K ratio in roots and exudate (From *Shone et al. [68]*).

Length of the root	Root: accumulation ratio	Exudate	
cm	Na/K	Volume ml/root	Na/K ratio
4	0.023	0.026	0.26
8	0.014	0.071	0.10
16	0.017	0.092	0.03

Presumably in some plant species like beans there exists an additional mechanism which prevents a sodium accumulation in the shoots: sodium is translocated again from the shoot to the root and is also given off to the external solution *(Barbier* and *Chabannes [3]; Cooil et al. [13]; Solovyov [70]; Brouwer* and *Levi [8]).* Differences in the accumulation of sodium in the shoot are therefore decisive for differences between plant species with respect to replacement of potassium by sodium. To answer the question however why sodium can replace potassium one has to consider the general and specific functions of potassium in metabolic processes in the plant.

As a general function the effect of potassium on the osmotic pressure of the cell sap can be considered. Cell elongation and turgescence of vacuolated cells are governed by soluble substances in the cell sap; inorganic ions and organic salts contribute mainly to this osmotic pressure of the cell. An example for this is shown in Table 3.

Table 3. Osmotic value in atm. of the most important compounds in cell sap of leaves of *Secale cereale* (From *Knodel [33]*).

Sugar	Inorganic salts	Organic salts	Organic acids
4.31	6.71	2.10	2.24

Investigations on 200 plant species by *Iljin* [30] showed that in 84% of the species the contribution of inorganic ions to the total osmotic pressure was more than half, in 63% of the species even more than ⅔. Extreme conditions exist in halophytes where more than 90% of the osmotic pressure is attributable to inorganic ions *(Steiner* [72]*)*. Normally in their effect on osmotic pressure among the cations in glycophytes potassium and in halophytes sodium are the most important. In glycophytes the concentration of potassium in the vacuole ranges between 50 and 200 mmol *(Pierce* and *Higinbotham* [57]*)*. In this nonspecific function within the vacuole potassium might be replaceable to a high extent. The pronounced beneficial effect of sodium on growth under conditions of insufficient supply of potassium might be mainly due to this replacement of potassium within the vacuole. This replacement can make potassium available for specific functions within the cell (vacuole ⟶ cytoplasm) as well as for retranslocation within the plant from older to younger leaves e.g. Such a retranslocation can be deduced from the results of *Coïc et al.* [11]; *El-Sheikh* and *Ulrich* [18], and *El-Sheikh et al.* [19] (Table 4). In plant species which hardly translocate sodium to the shoot (e.g. maize) at least a replacement of potassium within the root will take place and make this potassium available for translocation into the shoot. A very low potassium content in the roots is then the result of this replacement. This can be demonstrated in a comparison between maize and sugar beet plant (Table 5) under conditions of insufficient supply of potassium but high supply of sodium.

One can conclude that in its functions within the vacuole (osmotic effect; maintenance of the cation/anion-balance) potassium is presumably entirely replaceable by sodium because these functions are not specific and can be taken over by other cations like sodium. The extent of this replacement of potassium is dependent upon the rate by which sodium is taken up and translocated in relation to potassium.

Specific functions of potassium are the influence on charge and hydration and therefore on conformation of the protein part of enzyme molecules and as the result of this on the enzymatic reaction itself. This influence is reflected in stabilization of the enzyme conformation *(Sorger* and *Evans* [71], affecting the K_m-values, i.e. the affinity between enzyme and substrate *(Hiatt* [27]*)*, or in increasing the V_{max}-values, i.e. making more binding

Table 4. Effect of potassium and sodium on growth and content of potassium and sucrose in sugar-beet plants (From *El-Sheikh* and *Ulrich* [18]).

Treatment meq/l		Dry matter in g		meq K/100 g		Sucrose in storage roots
				young	old	
K^+	Na^+	Shoot	Storage root	blades	petioles	%
1	0	36	6.2	59	52	8.0
1	16	96	32.1	32	7	8.6
12	0	86	46.2	79	182	8.0
12	16	99	53.6	89	192	9.4

Table 5. Content of potassium and sodium (μeq/g fresh matter) in maize and sugar-beet plants after growth in nutrient solutions with 0.16 meq K^+ and 2.5 meq Na^+/l (From *Marschner* and *Schafarczyk* [43]).

	Roots		Shoots	
	K	Na	K	Na
maize	6.3	50.0	29.3	13.0
sugar-beet	28.0	15.5	52.0	51.5

sites available *(Evans et al. [21])*. These functions of potassium were reported in a review by *Evans* and *Sorger [20]*. For answering our question however it is only interesting to know if at all or to what extent potassium can be replaced by sodium in this functions. Several comparable experiments dealing with this question have been carried out. From these experiments however only limited conclusions can be drawn with respect to the extent of replacement, because in most cases only potassium alone or sodium alone were compared with each other. An example for this is demonstrated in the activation of the pyruvic kinase by univalent cations (Table 6).

Table 6. Influence of univalent cations on the pyruvic kinase activity from pea seeds. Relative values at maximum velocities (From *Miller* and *Evans [47]*).

K^+	Rb^+	NH_4^+	Na^+	Li^+
100	85	76	31	0

Similar results were obtained by *Latzko [38]* using bakers yeast and by *McCollum et al. [45]* using seeds of cucumber. The effect of sodium is small in comparison to potassium, but an effect is present. In contrast to this potassium is not replaceable at all by sodium in activating the acetic thiokinase from spinach leaves *(Hiatt* and *Evans [28])*.

Potassium also strongly activates the starch synthetase in higher plants *(Murata* and *Akazawa [52])*. A comparison between potassium and sodium on the activity of the starch synthetase from sweet potato is shown in Table 7. Compared with potassium and other univalent cations the effect of sodium is small. – But there are still many questions open for discussion, e.g. the existence of pronounced differences between plant species in the activation of the starch synthetase by potassium *(Murata* and *Akazawa [53])*. Presumably the existence of isoenzymes *(Momotani* and *Kato [48])* is of special importance in accounting for these differences. Investigations on isoenzymes are receiving increasing attention, isoenzymes are detectable not only between different plant species but also within the tissue of one species. For example from rice leaves 4 isoenzymes of the malic dehydrogenase have been separated by starch gel electrophoresis *(Rocha* and *Ting [62])*, and from rice seeds several isoenzymes of the α-amylase have been isolated *(Tanaka et al. [74])*. If the protein molecules of these isoenzymes have such different properties that they can be separated it might be expected that also the external conditions like ion environment have a different influence on their activity. It would be of special interest for example to investigate the effect of potassium and sodium on the activity of the starch synthetase from sugar beet leaves.

Table 7. The apparent K_A and V_{max} values for different univalent cations in the activation of starch synthetase from sweet corn. (From *Nitsos* and *Evans [54]*).

Cation	K_A m*M*	V_{max} m μmol ADP/hr/μg Protein
K^+	6	1.67
Rb^+	11	1.49
Cs^+	14	1.44
NH_4^+	19	1.54
Na^+	3	0.25

In enzymatic studies usually only single salts of potassium and sodium are compared. For answering the question of ability of sodium to replace potassium such experiments alone are not sufficient because there is no doubt that potassium is essential for metabolic processes and cannot be replaced entirely by sodium. For our question rather these experiments are of special interest in which the ratio potassium/sodium had been varied. Thus *Hansson* and *Kylin* [*23*] and *Kylin* and *Gee* [*34*] obtained very instructive results in activating the ATPase from plant tissue by potassium and sodium (Figures 2 and 3).
The highest activity of the ATPase from sugar beet roots (Figure 2) and *Avicennia* leaves (Figure 3) was not obtained by potassium or sodium alone but at certain combinations of potassium and sodium. The appearance of several peaks could perhaps be explained with the existence of isoenzymes or structural changes within the enzyme molecule.
The highest activity of this enzyme system in the presence of both potassium and sodium in sugar beet tissue is in agreement with the results of growth experiments with this plant species where the best growth and the highest sucrose content (Table 4) are also obtained in the presence of both cations.
To influence enzymatic reactions directly via charge and hydration of the protein molecule or more indirectly via water structure (*Alekseev* and *Abdurakhamanov* [*2*]) relativly high concentrations of potassium are necessary. These concentrations are 10^3 times higher than the substrate concentration (*Sorger* and *Evans* [*71*]). It is conceivable that in this ionic environment – in spite of the different properties of the potassium and sodium ions – a part of potassium is replaceable by sodium without significant influence on the protein conformation and therefore on the enzymatic reaction itself. In this case sodium could compensate an insufficient supply of potassium. In addition to this probably in higher plants (see Figures 2 and 3) there are enzyme systems or at least isoenzymes which show their highest activity in an ionic environment of both potassium and sodium and not of potassium alone.
Besides its ability to replace potassium partially sodium would have in this case an additional function namely as can be shown on its influence on growth and sucrose content of sugar beet plants even at high levels of potassium (Table 4). Sodium can however assume the role of an essential plant nutrient in the strict sense of the term only if certain enzymatic reactions take place in the presence of sodium only as it is the case in halophytes (see below).
In certain plant species sodium seems to be of special importance for the phosphate metabolism. This can bee seen from the activation of ATPase from sugar beet roots (Figure 2) or from roots of *Pinus taeda* (*McClurkin* and *McClurkin* [*44*]). This importance of sodium is most pronounced in algae: In *Ankistrodesmus* (*Simonis* and *Urbach* [*69*]; *Ullrich-Eberius* and *Simonis* [*78*]) for example the uptake of phosphate in light and dark is much higher in presence of sodium than in presence of potassium (Figure 4). This could have a connection with a possible special function of sodium in transport of phosphate through the membranes of these cells (*Ullrich-Eberius* and *Simonis* [*79*]; see also *Baumeister et al.* [*5*]). Using isolated mitochondria of *Brassica rapa* (*Shah* and *Wedding* [*67*]) were able to demonstrate that the esterification of phosphate and the quotient PO_4/O_2 were increased much more by sodium than by potassium. It is an open question if this beneficial influence on the phosphate metabolism has some connection with the formation of complexes with ATP; potassium might influence the phosphate metabolism by forming complexes with ATP (*Lowenstein* [*41*]; *Rechnitz* and *Mohan* [*61*]) and sodium is able to form more stable complexes with polyphosphates than potassium (*Lamm* and *Malmgren* [*35*]).

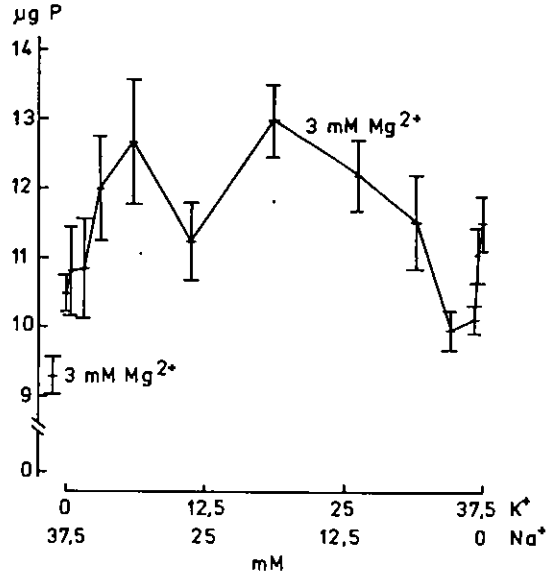

Figure 2. Effect of the sodium/potassium proportion on the ATPase-activity from sugar-beet roots. (From *Hansson* and *Kylin*, [*23*]).

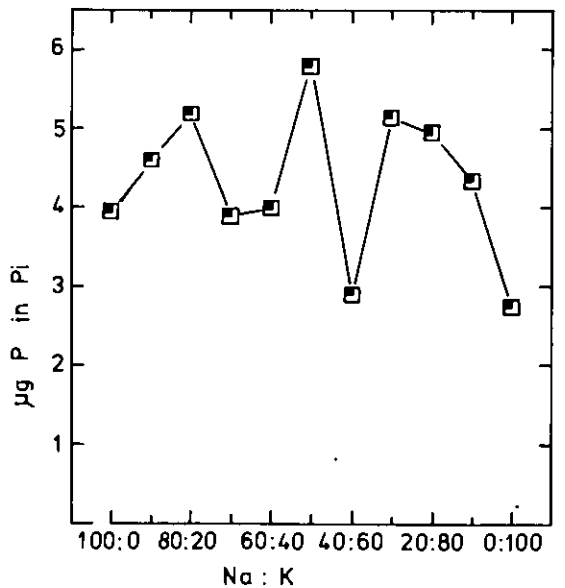

Figure 3. ATPase-activity of the microsomal fraction of *Avicennia* leaves at different proportions of sodium and potassium. (From *Kylin* and *Gee*, [*34*]).

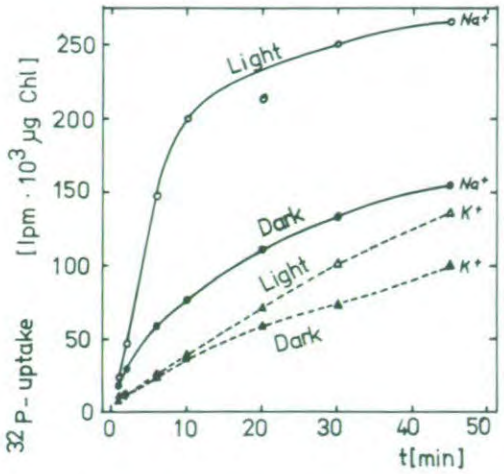

Figure 4. Uptake of phosphate by *Ankistrodesmus br.* in presence of potassium and sodium in light and dark, $1{,}88 \times 10^{-6}$ mol P/l, air = 3% CO_2. (From *Ullrich-Eberius* and *Simonis*, [78]).

Of special interest is the question of replacing potassium by sodium in the photosynthesis. The special function of potassium in this process was demonstrated by *Pirson* [58], the effect on the photophosphorylation is especially remarkable *(Latzko* and *Mechsner* [39]). As shown by *Dilley* and *Vernon* [15] and *Rumberg et al.* [63] in this case flux processes of potassium and H^+ through thylakoid membranes of the chloroplasts could be of special significance. Besides its function in activating the chloroplast enzymes potassium therefore seems to have additional functions within the chloroplast and so the high potassium content of these organelles is easy to understand.

As shown in Table 8 chloroplasts can also contain high concentrations of sodium (e.g. *Beta vulgaris*); in chloroplasts of *Limonium vulgare* the content of sodium is even higher than the content of potassium. Considering the beneficial effect of sodium in *Beta vulgaris* it is thinkable that besides potassium sodium participates in activating chloroplast enzymes and perhaps also in flux processes through the thylakoid membranes — and therefore in photophosphorylation — of this plant species. A prerequisite for this replacing function is a high membrane permeability for sodium. From the high mobility of sodium within the plant it may be concluded that in *Beta vulgaris* this prerequisite is fulfilled.

Without doubt, also in the chloroplasts the extent of replacement of potassium by sodium is mainly dependent on the plant sepcies. Indications for this are the pronounced changes

Table 8. Ion content of chloroplasts isolated by non-aqueous means from three plant species (From *Larkum [36]*).

	Ionic content (nmol/mg dry chloroplasts)				
	Na	K	Cl	Mg	Ca
Tolypella intricata	65	620	620	103	39
Beta vulgaris	390	500	130	220	26
Limonium vulgare	960	540	1250	200	168

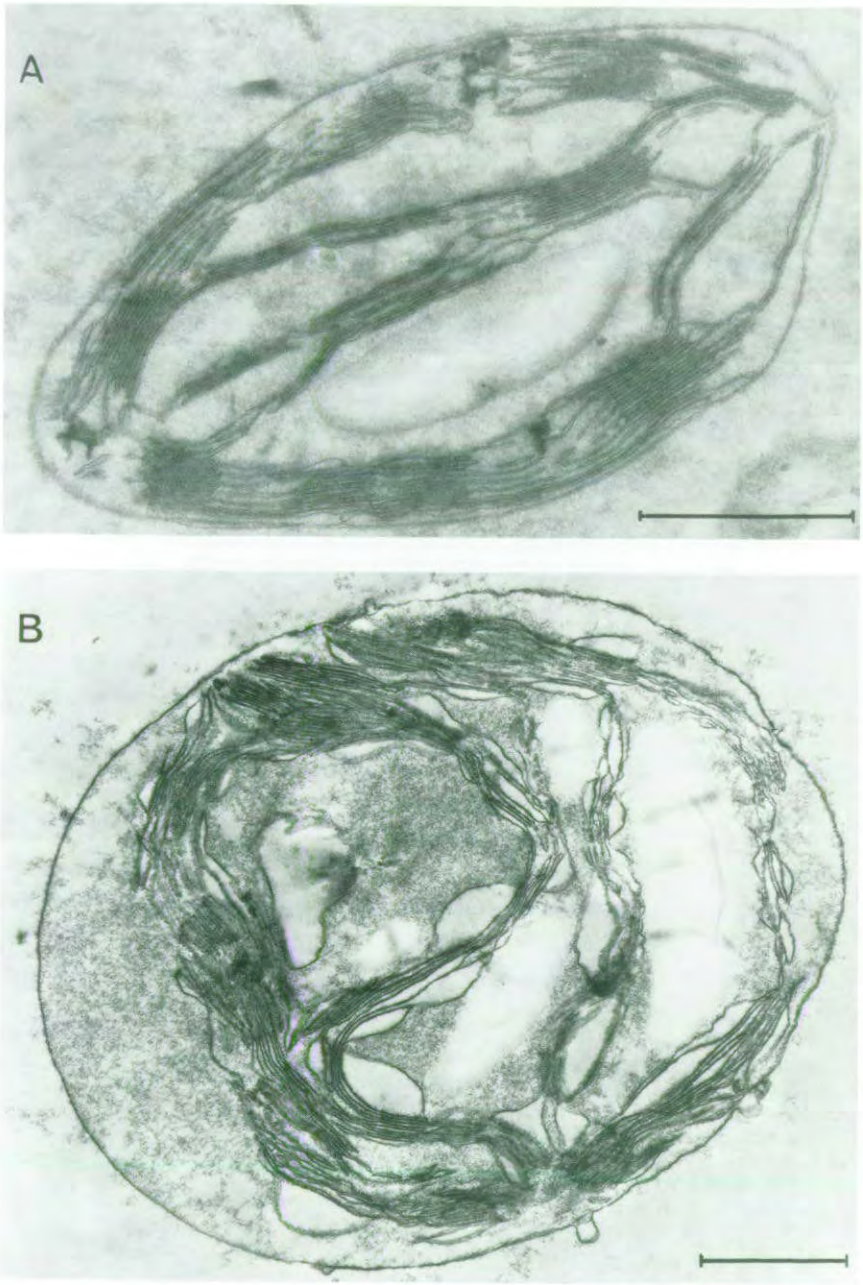

Figure 5. Effect of treatment of bean leaf segments for 8 hours with 25 meq KCl/l (A) or 25 meq NaCl/l (B) on the fine structure of the chloroplasts. (*G. Mix*, unpublished).

in the fine structure of the proplastids in cells of the maize root tips after exchange of potassium by sodium *(Hecht-Buchholz* and *Marschner [26])*. Maize belongs to those plant species which are sensitive against higher concentrations of sodium.

In chloroplasts of bean leaves also similar changes occur in the fine structure after exchanging potassium by sodium. As shown in Figure 5 after replacing of about 75% of the original potassium content of the leaf tissue by sodium there is a pronounced swelling of the chloroplasts. Either there are in the chloroplasts proteins which are sensitive against sodium or/and sodium is unable to participate in flux processes through the thylakoid membranes of this plant species. The sensitivity of bean plants to sodium is well known and preferential accumulation of sodium in the root and stem tissue of this plant species suggests a protective measure against high sodium concentrations in the leaves, especially in the chloroplasts.

The special function of potassium in the water economy of the higher plants is well known. As shown by *Peaslee* and *Moss [56]* this effect of potassium on water economy is at least partially caused by influencing the stomata movement. Under the influence of light potassium is translocated from the surrounding epidermal cells to the chlorophyllous stomata cells and increases in these cells the osmotic pressure and therefore the turgescence; this causes the stomata opening in light *(Fischer [22])*. In this function potassium is replaceable only to a small extent by sodium in leaf strips of *Vicia faba* (Figure 6). In this case however a generalization is not permitted because in leaf strips of *Commelina communis* sodium is even more effective in influencing the stomata opening than potassium (Table 9).

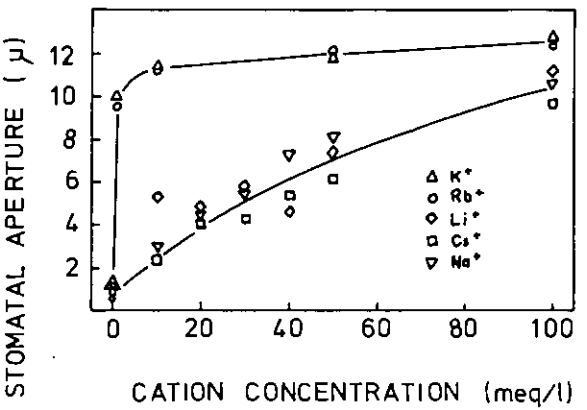

Figure 6. Stomatal opening of *Vicia faba* Epidermal strips in response to the cations (as chlorides) in light. (From *Humble* and *Hsiao, [29]*).

Table 9. Stomatal opening (µm) in light of isolated epidermis of *Commelina communis*, as affected by the concentration of KCl and NaCl in the incubation medium (From *Willmer* and *Mansfield [81]*).

	mol KCl or NaCl			
	0.0	0.1	0.2	0.3
KCl	0.0	3.5	5.5	5.9
NaCl	0.0	5.7	7.6	7.5

Prerequisite for this influence on stomata movement is a high permeability of the membrans to these ions. From the differences between the plant species with respect to the membrane permeability for potassium and sodium one can suppose that in plant species with high membrane permeability to sodium (e.g. *Beta vulgaris*) potassium is at least partially replaceable also in this function in the water economy of the plant.
There is also a suggestion that sodium plays a specific role in water economy of the plant. *Adriani* [*1*] and *Tullin* [*77*] found a remarkable reduction of the transpiration after supply of NaCl. Presumably this reduction is caused however by changing the leaf anatomy towards succulence by chloride *(Meiri* and *Poljakoff-Mayber* [*46*]*)* and is not a specific effect of sodium.
A high concentration and mobility of potassium in the phloem as well as the continuous circulation within the plant (e.g. potassium is translocated from the shoot to the root even at high supply of potassium in the external solution) are indications for a special function of potassium in long distance transport processes in higher plants. *Dijkshoorn et al.* [*14*] developed the interesting conception that potassium is retranslocated from the shoot into the root as counterion of organic acid anions. After degradation of the organic acid anion in the roots potassium is available as a counterion for nitrate which is taken up from the external solution and both potassium and nitrate are translocated together to the shoot. After nitrate reduction in the shoot and the production of an equivalent amount of organic acid anions *(Ben-Zioni et al.* [*6*]*)* this retranslocation can be repeated. This function of potassium as a counterion in long distance transport should not be specific and other cations should be able to replace potassium in this function provided they are mobile in the phloem. In comparative experiments with maize and sugar beet (Table 10) it was obvious that even at higher external supply of both sodium and potassium a retranslocation of sodium from the maize shoot is not dedectable. In contrast to maize in sugar beet the influx of sodium into the shoot (2.3 μeq) is nearly equal to the efflux of sodium from the shoot in direction to the root (2.2 μeq) and even higher than the simultaneous efflux of potassium (0.9 μeq). Therefore in sugar beet there is a similar circulation of sodium within the plant just as the circulation of potassium in other plant species. It can be concluded that in this plant species sodium can replace potassium to a high extent in its function in long distance transport, e.g. nitrate translocation.
In addition to its ability to replace potassium more or less in some plant species sodium has also specific functions in certain species and there represents an essential plant nutrient. This can be demonstrated most clearly in halophytes *(Keller* [*32*]*; Brownell* and *Wood* [*9*], e.g. *Atriplex vesicaria* (Table 11). In the absence of sodium the growth ceases and the plants show pronounced sodium deficiency symptoms. These symptoms disappear only after addition of sodium. Immediately after addition of sodium respiration and chlorophyll content increase, followed by an increase in growth rate *(Brownell* and *Jackman* [*10*]*).* Potassium cannot replace sodium in this plant species.

Table 10. Flux of potassium and sodium in shoots of maize and sugar-beet plants. External solution 10 meq KCl + 10 meq NaCl. Values in μeq/g fresh matter per 4 hours (From *Marschner* and *Schafarczyk [43]*).

	Potassium		Sodium	
	Maize	Sugar-beet	Maize	Sugar-beet
Influx	1.8	2.7	0.7	2.3
Efflux	0.2	0.9	0.0	2.2

Table 11. Effect of sodium on the growth of *Atriplex vesicaria* in water culture (From *Brownell* and *Wood [9]*).

Treatment meq/l as sulfates	Dry matter/vessel after 48 days
0.00	0.056
0.02 Na	0.340
0.10 Na	0.513
0.60 Na	0.493
0.60 K	0.066

It is also typical for *Atriplex* species that they not only show better growth in presence of sodium *(Mozafar et al. [40])* but also take up sodium in preference to potassium (see also the reserve of dual mechanism in uptake in the mangrove *Avicennia marina; Rains* and *Epstein [60]*). In addition in these plant species sodium competes with potassium but potassium does not compete with sodium during uptake *(Mozafar et al. [50])*. Without doubt in this genus sodium has specific and essential functions.

There are still many questions open with respect to potassium replacement by sodium. More attention to differences between plant species, consideration of the existence of isoenzymes even within a cell, and modified studies also in enzymatic investigations (variation of the ratio potassium/sodium instead of using single salt solutions) will presumably enable us to give a more precise answer to the question: Why can sodium replace potassium in plants?

Bibliography

1. *Adriani, M.J.:* Sur la transpiration de quelques halophytes cultivées dans des milieux différents en comparaison avec celle de quelques non-halophytes. Proc. Kon. Ned. Akad. v. Wetenesch. *40*, 524–529 (1937).
2. *Alekseev, A.M.*, and *Abdurakhamanov, A.A.:* On the influence of ions of mineral nutrient salts on the state of the protoplasm of plant cells. Fiziol. Rast. *13*, 414–419 (1966).
3. *Barbier, G.*, and *Chabannes, J.:* Accumulation du sodium dans les racines des plantes. Comptes R. Acad. Sci. *232*, 1372–1374 (1951).
4. *Baumeister, W.:* Das Natrium als Pflanzennährstoff. Gustav Fischer Verlag, Stuttgart 1960.
5. *Baumeister, W., Bade, A.*, and *Conrad, D.*: Die physiologische Bedeutung des Natriums für die Pflanze. II. Versuche mit niederen Pflanzen. (Forschungsber. d. Landes Nordrhein-Westf. Hrsg. von Leo Brandt. Nr. 1678.) Köln, Opladen: Westdeutsch. Vlg.,1966.
6. *Ben-Zioni, A., Vaadia, Y.*, and *Lips, S.H.:* Correlations between nitrate reduction, protein synthesis and malate accumulation. Physiol. Plant. *23*, 1039–1047 (1970).
7. *Bower, C.A.*, and *Wadleigh, C.H.:* Growth and cationic accumulation by four species of plants as influenced by various levels of exchangeable sodium. Soil Sci. Soc. Amer. Proc. *13*, 218–223 (1949).
8. *Brouwer, R.*, and *Levi, E.:* Responses of bean plants to root temperatures. IV. Translocation of ^{22}Na applied to the leaves. Acta Bot. Neerl. *18*, 58–66 (1966).
9. *Brownell, P.F.*, and *Wood, J.G.:* Sodium as an essential micronutrient element for *Atriplex vesicaria* Heward. Nature *179*, 365–636 (1957).
10. *Brownell, P.F.*, and *Jackman, M.E.:* Changes during recovery from sodium deficiency in *Atriplex*. Plant Physiol. *41*, 617–622 (1966).
11. *Coïc, Y., Lesaint, C.*, and *Piollat, M.-T.:* Influence de la déficience en eau sur la composition minérale de l'orge. Ann. Physiol. veg. *4*, 227–234 (1962).
12. *Collander, R.:* Selective absorption of cations by higher plants. Plant Physiol. *16*, 691–720 (1941).
13. *Cooil, B.J., de la Fuente, R.K.* and *de la Pena, R.S.:* Absorption and transport of sodium and potassium in squash. Plant Physiol. *40*, 625–632 (1965).
14. *Dijkshoorn, W., Lathwell, D.D.*, and *De Wit, C.T.:* Temporal changes in carboxylate content of ryegrass with stepwise changes in nutrition. Plant and Soil *29*, 369–390 (1968).
15. *Dilley, R.A.*, and *Vernon, L.P.:* Ion and water transport processes related to light-dependent shrinkage of spinach chloroplasts. Arch. Biochem. Biophys. *111*, 365–375 (1965).
16. *Dorph-Peterson, K.*, and *Steenbjerg, F.:* Investigations of the effect of fertilizers containing sodium. Plant and Soil *2*, 283–300 (1950).
17. *Draycott, A.P., Marsh, J.A.P.*, and *Tinker, P.B.H.:* Sodium and potassium relationship in sugar beet. J. agric. Sci. *74*, 567–573 (1970).

18. *El-Sheikh, A.M.*, and *Ulrich, A.*: Interactions of rubidium, sodium, and potassium on the nutrition of sugar beet plants. Plant Physiol. *46*, 645–649 (1970).
19. *El-Sheikh, A.M., Ulrich, A.*, and *Broyer T.C.*: Sodium and rubidium as possible nutrients for sugar beet plants. Plant Physiol. *42*, 1202–1208 (1967).
20. *Evans, H.J.*, and *Sorger, G.J.*: Role of mineral elements with emphasis on the univalent cations. Ann. Rev. Plant Physiol. *17*, 47–76 (1966).
21. *Evans, H.J., Clark, R.B.*, and *Russel, S.A.*: Cation requirements for the acetic thiokinase from yeast. Biochim. Biophys. Acta *92*, 582–594 (1964).
22. *Fischer, R.A.*: Stomatal opening: Role of potassium uptake by guard cells. Science *160*, 784–785 (1968).
23. *Hansson, G.*, and *Kylin, A.*: ATP-ase activities in homogenates from sugar-beet roots, relation to Mg^{++} and $(Na^+ + K^+)$-stimulation. Z. Pflanzenphysiol. *60*, 270–275 (1969).
24. *Harmer, P.M.*, and *Benne, E.J.*: Sodium as a crop nutrient. Soil Sci. *60*, 137–148 (1945).
25. *Harmer, P.M., Benne, E.J., Laughlin, W.M.*, and *Key, C.*: Factors affecting crop response to sodium applied as common salt on Michigan muck soil. Soil Sci. *76*, 1–17 (1953).
26. *Hecht-Buchholz, Ch.*, and *Marschner, H.*: Veränderungen der Feinstruktur von Zellen der Maiswurzelspitze bei Entzug von Kalium. Z. Pflanzenphysiol. *63*, 416–427 (1970).
27. *Hiatt, A.J.*: Formic acid activation in plants: II. Activation of formyltetrahydrofolate synthetase by magnesium, potassium, and other univalent cations. Plant Physiol. *40*, 189–193 (1965).
28. *Hiatt, A.J.*, and *Evans, H.J.*: Influence of certain cations on activity of acetic thiokinase from spinach leaves. Plant Physiol. *35*, 673–677 (1960).
29. *Humble, G.D.*, and *Hsiao, T.C.*: Specific requirement of potassium for light-activated opening of stomata in epidermal strips. Plant Physiol. *44*, 230–234 (1969).
30. *Iljin, W.S.*: Zusammensetzung der Salze in der Pflanze auf verschiedenen Standorten. Kalkpflanzen. Beih. bot. Zbl. *50*, 95–137 (1932).
31. *Jacoby, B.*: Sodium retention in excised bean stems. Physiol. Plant. *18*, 730–739 (1965).
32. *Keller, B.*: Halophyten- und Xerophytenstudien. J. Ecology *13*, 225–255 (1925).
33. *Knodel, H.*: Eine Methodik zur Bestimmung der stofflichen Grundlagen des osmotischen Wertes von Pflanzenpreßsäften. Planta *28*, 704–715 (1939).
34. *Kylin, A.*, and *Gee, R.*: Adenosine triphosphatase activities in leaves of the mangrove *Avicennia nitida* Jacq. Plant. Physiol. *45*, 169–172 (1970).
35. *Lamm, O.*, and *Malmgren, H.*: Measurements of the degree of dispersion of a highly polymerized metaphosphate prepared according to Tammann. Z. Anorg. u. allgm. Chem. *245*, 103–120 (1940).
36. *Larkum, A.W.D.*: Ionic relations of chloroplasts *in vivo*. Nature *218*, 447–449 (1968).
37. *Larson, W.E.*, and *Pierre, W.H.*: Interaction of sodium and potassium on yield and cation composition of selected crops. Soil Sci. *76*, 51–64 (1953).
38. *Latzko, E.*: Zur Wirkung von Alkaliionen auf den aeroben Hexoseabbau. Z. Pflanzenernährung, Düng. u. Bdk. *111*, 38–52 (1965).
39. *Latzko, E.*, and *Mechsner, K.*: Bedeutung von Alkali-Ionen für die Intensität der Lichtphosphorylierung bei *Chlorella vulgaris*. Naturwiss. *45*, 247–248 (1958).
40. *Lehr, J.J.*: Sodium as a plant nutrient. J. of the Science of Food and Agriculture *4*, Nr. 20, 460–468 (1953).
41. *Lowenstein, J.M.*: The stimulation of transphosphorylation by alkalimetal ion. Biochem. J. *75*, 269–274 (1960).
42. *Marschner, H.*, and *Schafarczyk, W.*: Vergleich der Nettoaufnahme von Natrium und Kalium bei Mais- und Zuckerrübenpflanzen. Z. Pflanzenernähr. u. Bodenk. *118*, 172–187 (1967).
43. *Marschner, H.*, and *Schafarczyk, W.*: Influx und Efflux von Natrium und Kalium bei Mais- und Zuckerrübenpflanzen. Z. Pflanzenernähr. u. Bodenk. *118*, 187–201 (1967).
44. *McClurkin, I.T.*, and *McClurkin, D.C.*: Cytochemical demonstration of a sodium-activated and a potassium-activated adenosine-triphosphatase in Loblolly pine seedling root tips. Plant Physiol. *42*, 1103–1110 (1967).
45. *McCullum, R.E., Hagemann, R.H.*, and Tyner, E.H.: Influence of potassium on pyruvic kinase from plant tissue. Soil Sci. *86*, 324–331 (1958).
46. *Meiri, A.*, and *Poljakoff-Mayber, A.*: The effect of chlorine salinity on growth of bean-leaves in thickness and in area. Israel J. Bot. *16*, 115–123 (1967).
47. *Miller, G.W.*, and *Evans, H.J.*: The influence of salts on pyruvic kinase from tissues of higher plants. Plant Physiol. *32*, 346–354 (1957).
48. *Momotani, Y.*, and *Kato, J.*: Isoenzymes of α-amylase induced by gibberellic acid in embryoless grains of barley. Platn Physiol. *41*, 1395–1396 (1966).
49. *Montasier, A.H., Sharoubeem, H.H.*, and *Sidrak, G.H.*: Partial substitution of sodium for potassium in water cultures. Plant and Soil *25*, 181–194 (1966).
50. *Mozafar, A., Goodin, J.R.*, and *Oertli, J.J.*: Na- and K-interactions in increasing the salt tolerance of *Atriplex halimus* L.I. Yield characteristic and osmotic potential. Agr. J. *62*, 478–481 (1970).
51. *Mozafar, A., Goodin, J.R.*, and *Oertli, J.J.*: Sodium and potassium interactions in increasing the salt tolerance of *Atriplex haltmus*. II. Na^+ and K^+ uptake characteristics. Agr. J. *62*, 481–484 (1970).
52. *Murata, T.*, and *Akazawa, T.*: Enzymic mechanism of starch synthesis in sweet potato roots. I. Requirement of potassium ions for starch synthetase. Arch. Biochem. u. Biophys. *126*, 873–879 (1968).
53. *Murata, T.*, and *Akazawa, T.*: Stimulative effect of potassium ion on starch synthetase of different plant origins. Plant and Cell Physiol. *10*, 457–560 (1969).

54. *Nitsos, R.E.*, and *Evans, H.J.*: Effects of univalent cations on the activity of particulate starch synthethase. Plant Physiol. *44*, 1260–1266 (1969).
55. *Pearson, G.A.*: Absorption and translocation of sodium in beans and cotton. Plant Physiol. *42*, 1171–1175 (1967).
56. *Peaslee, D.E.*, and *Moss, D.N.*: Photosynthesis in K- and Mg-deficient maize (*Zea mays* L.) leaves. Soil Sci. Soc. Proc. Amer. *30*, 220–223 (1966).
57. *Pierce, W.S.*, and *Higinbotham, N.*: Compartments and fluxes of K^+, Na^+ and Cl^- in *Avena coleoptile* cells. Plant Physiol. *46*, 666–673 (1970).
58. *Pirson, A.*: Ernährungs- und stoffwechselphysiologische Untersuchungen an Frontalis und Chlorella. Z. Bot. *31*, 193–267 (1937).
59. *Rains, D.W.*: Sodium and potassium absorption by bean stem tissue. Plant Physiol. *44*, 547–554 (1969).
60. *Rais, D.W.*, and *Epstein, E.*: Preferential absorption of potassium by leaf tissue of the mangrove, *Avicennia marina*: an aspect of halophylic compentence in coping with salt. Austr. J. Biol. Sci. *20*, 847–857 (1967).
61. *Rechnitz, G.A.*, and *Mohan, M.S.*: Potassium-adenosine triphosphate complex: Formation constant measured with ion selective electrodes. Science *168*, 1460 (1970).
62. *Rocha, V.*, and *Ting, I.P.*: Tissue distribution of microbody, mitochondrial, and soluble malate dehydrogenase isoenzymes. Plant Physiol. *46*, 754–756 (1970).
63. *Rumberg, B., Reinwald E., Schröder, H.*, and *Sippel, U.*: Correlation between electron flow, proton translocation and phosphorylation in chloroplasts. Naturwiss. *55*, 77–79 (1968).
64. *Saalbach, E.*, and *Aigner, H.*: Über die Wirkung einer Natriumdüngung auf Natriumgehalt, Ertrag und Trockensubstanzgehalt einiger Gras- und Kleearten. Landw. Forsch. *23*, 264–274 (1970).
65. *Scharrer, K.*, and *Kühn, H.*: Die Wirkung des Natriums im Rhenaniaphosphat. Z. Pflanzenernähr., Düng. u. Bodenk. *80*, 189–202 (1958).
66. *Scharrer, K., Schreiber, R.*, and *Kühn, H.*: Über den Einfluss des Natriums auf das Wachstum von Futter- und Zuckerrüben bei ausreichender Versorgung mit Kalium. Z. Pflanzenernähr., Düng. u. Bodenk. *62*, 128–137 (1953).
67. *Shah, B.H.*, and *Wedding, R.T.*: Sodium ion influence on phosphorylations associated with oxidation of succinate by turnip root mitochondria. Science *160*, 304 306 (1968).
68. *Shone, M.G.T., Clarkson, D.T.*, and *Sanderson, J.*: The absorption and translocation of sodium by maize seedlings. Planta (Berl.) *86*, 301–314 (1969).
69. *Simonis, W.*, and *Urbach, W.*: Über eine Wirkung von Natrium-Ionen auf die Phosphataufnahme und die lichtabhängige Phosphorylierung von *Ankistrodesmus braunii*. Archiv f. Mikrobiol. *46*, 265–286 (1963).
70. *Solovyov, V.A.*: Effect of high concentrations of NaCl on potassium and sodium uptake and distribution in separately fed pumpkin plants. Fiziol. Rastenij *13*, 320-326 (1966).
71. *Sorger, G.J.*, and *Evans, H.J.*: Effect of univalent cations on the properties of yeast NAD + acetaldehyde dehydrogenase. Biochim. Biophys. Acta *118*, 1–8 (1966).
72. *Steiner, A.*: Die Zusammensetzung des Zellsaftes bei höheren Pflanzen in ihrer ökologischen Bedeutung. Erg. d. Biologie *17*, 151–254 (1939).
73. *Stoklasa, J.*: Über die Abhängigkeit der Resorption des Kaliumions von der Gegenwart des Natriumions im Organismus der Zuckerrübe. Biochem. Ztschr. *73*, 260–312 (1916).
74. *Tanaka, Y., Ito, T.*, and *Akazawa, T.*: Enzymatic mechanism of starch breakdown in germinating rice seed. III. α-Amylase isoenzymes. Plant Physiol. *46*, 650–654 (1970).
75. *Tinker, P.B.H.*: The effects of nitrogen, potassium and sodium fertilizers on sugar beet. J. Agric. Sci. *65*, 207–212 (1965).
76. *Truog, E., Berger, K.C.*, and *Attoe, O.J.*: Response of nine economic plants to fertilization with sodium. Soil Sci. *76*, 41–50 (1953).
77. *Tullin, V.*: Response of sugar beet to common salt. Physiol. Plant. *7*, 810–834 (1954).
78. *Ullrich-Eberius, C.I.*, and *Simonis, W.*: Der Einfluss von Natrium- und Kaliumionen auf die Phosphataufnahme bei *Ankistrodesmus braunii*. Planta *93*, 214–226 (1970).
79. *Ullrich-Eberius, C.I.*, and *Simonis, W.*: Der Einfluss von Natrium- und Kaliumionen auf die Photophosphorylierung bei *Ankistrodesmus braunii*. Planta *92*, 358–373 (1970).
80. *Wallace, A., Hemaidan, N.*, and *Sufii, S.M.*: Sodium translocation in bush beans. Soil Sci. *100*, 331–334 (1965).
81. *Willmer, C.M.*, and *Mansfield, T.A.*: Active cation transport and stomatal opening, a possible physiological role of sodium ions. Z. Pflanzenphysiol. *61*, 398–400 (1970).
82. *Wybenga, J.M.*: A contribution to the knowledge of the importance of sodium for the plant life. Dissertation Wageningen 1957.

Transport of Sodium and Potassium, and Properties of (Sodium + Potassium)-Activated Adenosine Triphosphatases: Possible Connection with Salt Tolerance in Plants

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Summary

An extrusion mechanism for Na^+ was demonstrated in the unicellular green alga *Scenedesmus*. Sodium had also an influence on the uptake and retention of rubidium (and, by inference, potassium). This led us to a search for $(Na^+ + K^+)$-activated adenosine triphosphatases in plants. They were demonstrated in sugar beet and in the mangrove *Avicennia nitida*. Further work, presented in this paper, compares the salt accumulation of inbred lines of sugar beet with the properties of their $(Na^+ + K^+)$-activated ATPases. High- and low-salt lines show only one peak each, with different sodium-potassium requirements for the two types. Intermediate lines show double peaks for activation. To the extent that electrochemical data are available for vacuolated plant cells, they indicate two mechanisms for extrusion of Na^+ from the cytoplasm: the one in the plasmalemma, coupled to uptake of K^+; the other in the tonoplast. Furthermore, salt tolerance in vacuolated plants seems to be correlated with a high ratio of Na:K, contrary to what is found in nonvacuolated cells. It is argued that the occurence of two transport systems for sodium could give the salt tolerant plants maximum protection of the cytoplasm against Na^+, at the same time as sodium can be utilized as an osmotic agent in the vacuole. The two activity peaks in our material may represent protection at the levels of the plasmalemma and the tonoplast respectively.

1. *Introduction*

Extrusion of sodium has been recognized as a key process in animal cells and tissues, where it occurs coupled to uptake of potassium and, biochemically, reveals itself as $(Na^+ + K^+)$-activated ATPases (review by *Skou* [*12*]). Contrary to this, the classical view of plant physiology was that the control of sodium transport is to be found in uptake processes. However, electrochemical measurements indicated that sodium extrusion could be of importance in plants, although it was at that time not possible to exclude permeability barriers during the uptake as the main points of control (review by *Dainty* [*2*]). We became interested in these questions, when we found that the unicellular green alga *Scenedesmus* can be manipulated so that it reverts from sodium uptake to sodium extrusion *(Kylin* [*7*]*)*. The presence of sodium in the medium has a regulating influence on the uptake and retention of rubidium (and, by inference, potassium) and cesium *(Kylin* [*8*]*)*.

With the background given above, it seemed logical to try to find $(Na^+ + K^+)$-activated ATPases in plants, where they had so far not been detected. From the preparative point of view, *Scenedesmus* was not suitable for the purpose. It was decided that sugar beet should represent a fair chance to achieve the goal. It is well known that the dry weight of the beets is increased by adding sodium to the fertilizer. Lack of sodium leads to symptoms similar to those of K deficiency *(El-Sheik et al.* [*3*]*)*. It was tempting to think of this sodium effect as connected with influences on potassium transport, a combination which might lead to a comparatively good chance to find the ATPases.

The data of *Van Steveninck* [15] on how tris influences the transport of potassium in beet tissue made us look for other buffer systems. Successful results to prepare homogenates with ($Na^+ + K^+$)-activated ATPase properties were obtained when roots of beet seedlings were homogenised in 0.03 *M* histidine-HCl at pH 7.2 with 0.20 *M* sucrose as osmoticum. The optima for pH and Mg^{2+} were determined for the fraction taken between 2500 *g* and 20000 *g* and treated for 1 hour with 0.1% deoxycholate. We could then demonstrate that the ATPase activity was further increased by monovalent cations and varied with the ratio of Na:K at a given constant level of ($Na^+ + K^+$) *(Hansson* and *Kylin* [4]).
Animal systems usually show only one peak of activation, but the beet ATPase was activated at two different ratios of Na:K. In homogenates of the saltexcreting leaves of the mangrove Avicennia nitida, an ATPase with activity peaks at 3 different ratios of Na:K has been demonstrated *(Kylin* and *Gee* [9]). Technically, these findings, together with the effect of varying salt concentrations, may explain the difficulties that have met in various attempts to find ($Na^+ + K^+$)-activated ATPases in plants *(Kylin* and *Gee* [9]).
Theoretically, the multiple ratios of Na:K for activation may be explained in different manners, for instance as allosteric transformations (cf. *Rothstein* [11]) or as the presence of different types of ATPases in different types of membranes in the homogenate. Some support for the latter possibility has been given by separations on sucrose gradients (*Jan Karlsson,* unpublished).

2. *Correlation between field properties and ATPase activation*

For research purposes, Dr. *O. Bosemark* of the *Swedish Sugar Company* has produced a selection of inbred lines of sugar-beet, with different properties as regards salt uptake in the field. These strains range from (low salt, high proportion of potassium) to (high salt, high proportion of sodium) varieties. Seedlings of these strains have been produced under uniform conditions in climate chambers, and the ATPase properties of root homogenates have been tested under a variety of Na:K ratios at different total ($Na^+ + K^+$) concentrations (*G. Hansson,* in preparation). The main data from these experiments are collected in Figures 1 to 4.
In this series of figures, (1) represents the (low salt, high proportion of potassium) extreme, with (2) and (3) successively leading over to the (high salt, high proportion of sodium) type in (4). The Na^+ dependency of the ATPase is represented by the distance between the two curves, which denote the highest and lowest activity at the K^+ concentration given by the abscissa.
The extremes give only one peak of activation each. The peaks are exhibited at extremely different combinations of sodium and potassium. The ATPases from the intermediate lines show two peaks each – just as the normal field variety – and in this pattern there is a gradual change from the one extreme to the other.

3. *Discussion*

To form a hypothesis on the biological implications of our findings, it may be useful to recall that the protection of cytoplasm against overhydratation may be one of the primary functions of the extrusion of sodium ions with their swelling activity *(Rothstein* [11]). Observations by *Norkrans* and *Kylin* [10] fit with this general wiew. Their data indicate a

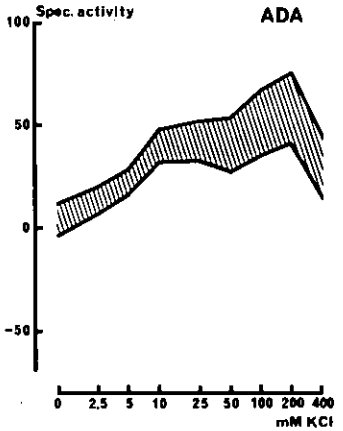

Figure 1. The ($Na^+ + K^+$) influence on the ATPase activities of root homogenates from the (low salt, high proportion of potassium) inbred line ADA (M 15/10/18). — *Abscissa:* Concentration of KCl in reaction medium (logarithmic scale). *Ordinate:* Specific activity expressed as nmoles P_i released per mg protein and minute. The activity obtained in the basic medium of 0.75 m*M* ATP and 3 m*M* $MgCl_2$ in 40 m*M* histidine-CHl buffer at pH 6.75 is taken as zero value. All tests run in duplicate at 30°C. Experiments were repeated with independent batches of beet seedlings. *The upper and lower line* denote the highest and lowest activity obtained with concentrations of 0, 2.5, 5, 10, 25, 50, 75, and 100 m*M* NaCl mixed with the KCl concentrations indicated. The shading thus indicates the sodium influence.

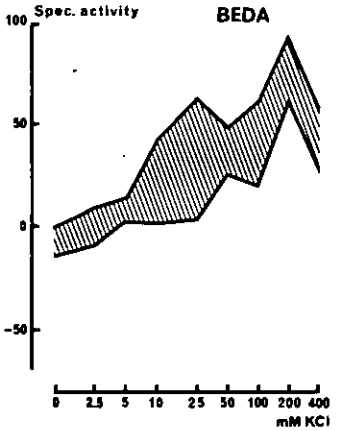

Figure 2. As Figure 1 for the intermediate line BEDA (G 114/10/12).

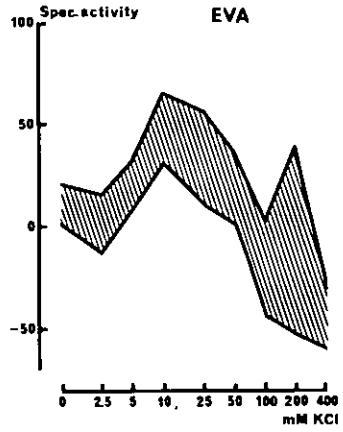

Figure 3. As Figure 1 for the intermediate line EVA (M 25/4/23).

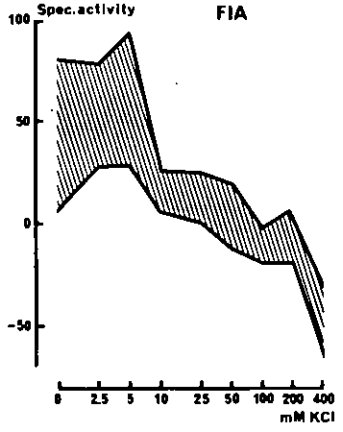

Figure 4. As Figure 1 for the (high salt, high proportion of sodium) inbred line FIA (F 183/1/18).

stronger uptake of K^+ and extrusion of Na^+ in the salt tolerant yeast *Debaryomyces* than in normal *Saccharomyces*, where the uptake of K^+ rather seems connected with excretion of H^+. As a consequence, the salt tolerant yeast has a higher ratio of K:Na than the non-tolerant cells.

However, in higher plants with their great central vacuoles, the opposite was reported by *Collander* [*1*]. As a rule, halophytes showed low ratios of K:Na, whereas the ratio was high in non-tolerant species. The highest and lowest levels of K^+ varied only by a factor of 2 to 3, where the Na^+ variation was by a factor of 20 to 60 when the different species were compared.

It seems possible to reconcile the apparent conflict between the data from yeast and from higher plants by remembering that protection of the cytoplasm against Na^+ cannot be a sufficient prerequisite for salt tolerance in higher plants, where the cells contain large central vacuoles. Such cells must also have a mechanism to beware their turgidity under osmotic stress.

The double prerequisite of protecting the cytoplasm against Na^+ and maintaining the turgor could be achieved by a double mechanism for the extrusion of Na^+, the one situated in the plasmalemma and the other in the tonoplast. When the plasmalemma mechanism cannot alone keep the sodium ions out of the cytoplasm, sodium would be translocated into the vacuole by the tonoplast pump. In this concept, sodium will be utilized as an osmotic agent, at the same time as maximum protection of the cytoplasm is obtained. Refined techniques have given electrochemical evidence for sodium extrusion from the cytoplasm at both the plasmalemma and the tonoplast levels (cf. *Jennings* [*6*]), in characean algae *(Spanswick* and *Williams* [*14*], *Spanswick et al.* [*13*]) as well as in higher plants *(Higinbotham* [*5*]).

Within this framework, it is tempting to regard the (low salt, high proportion of potassium) line ADA to have been obtained through a selection mainly for a transport ATPase localized in the plasmalemma, whereas the (high salt, high proportion of sodium) line FIA should chiefly contain a system localized in the tonoplast. The intermediate forms BEDA and EVA may contain both systems in various proportions or with various affinities for K^+ and Na^+.

This work has been pursued with the aid of grants from the *Swedish Council for Forestry* and *Agricultural Research*. The technical assistance of Miss *Gun Björklund* is gratefully acknowledged.

Bibliography

1. *Collander, R.*: Selective absorption of cations by higher plants. Plant Physiol. *16*, 691–720 (1941).
2. *Dainty, J.*: Ion transport and electrical potentials in plant cells. Ann. Rev. Plant Physiol. *13*, 379–402 (1962).
3. *El-Sheik, A.M., Ulrich, A.*, and *Broyer, T.C.*: Sodium and rubidium as possible nutrients for sugar-beet plants. Plant Physiol. *42*, 1202–1208 (1967).
4. *Hansson, G.*, and *Kylin, A.*: ATPase activities in homogenates from sugar-beet roots, relation to Mg^{2+} and ($Na^+ + K^+$)-stimulation. Z. Pflanzenphysiol. *60*, 270–275 (1969).
5. *Higinbotham, N.*: Movement of ions and electrogenesis in higher plant cells. Am. Zoologist *10*, 393–403 (1970).
6. *Jennings, D.H.*: Halophytes, succulence and sodium in plants – a unified theory. New Phytol. *67*, 899–911 (1968).
7. *Kylin, A.*: An outpump balancing phosphate dependent sodium uptake in Scenedesmus. Biochem. Biophys. Res. Commun. *16*, 497–500 (1964).
8. *Kylin, A.*: Uptake and loss of Na^+, Rb^+, and Cs^+ in relation to an active mechanism for extrusion of Na^+ in *Scendesmus*. Plant Physiol. *41*, 579–584 (1966).
9. *Kylin, A.*, and *Gee, R.*: Adenosine triphosphatase activities in leaves of the mangrove *Avicennia nitida* Jacq. Influence of sodium to potassium ratios and salt concentrations. Plant Physiol. *45*, 169–172 (1970).
10. *Norkrans, B.*, and *Kylin, A.*: Regulation of the potassium to sodium ratio and of the osmotic potential in relation to salt tolerance in yeast. J. Bact. *100*, 836–845 (1969).
11. *Rothstein, A.*: Membrane function and physiological activity of microorganisms. In: *J.F. Hoffman* (ed.): The Cellular Functions of Membrane Transport. pp. 23–39. Prentice-Hall, Englewood Cliffs, N.J., 1964.
12. *Skou, J.C.*: Enzymatic aspects of active linked transport of Na^+ and K^+ through the cell membrane. Progr. Biophys. Mol. Biol. *14*, 131–166 (1964).
13. *Spanswick, R.M., Stolarek, J.*, and *Williams, E.J.*: The membrane potential of Nitella translucens. J. Exp. Bot. *18*, 1–16 (1967).
14. *Spanswick, R.M.*, and *Williams, E.J.*: Electrical potentials and Na, K, and Cl concentrations in the vacuole and cytoplasm of *Nitella translucens*. J. Exp. Bot. *15*, 193–200 (1964).
15. *Van Steveninck, R.F.M.*: Potassium fluxes in red beet tissue during its 'lag phase'. Physiol. Plantarum *15*, 211–215 (1962.)

Discussion, Session No. 1

Dr. *T.Z. Nowakowski* (Harpenden/England):

Because it seems that both potassium and sodium are required for ATPase activity, does it follow that sodium is an essential nutrient?

Prof. *H.J. Evans* (Corvallis/USA):

Apparently (Na + K) ATPase has been identified only in a few species of higher plants. If it is established that Na + K ATPase is generally distributed in and is functional in higher plants then such information would suggest that Na^+ is an essential element. Other information however would be required for proof of essentiality.

Prof. Dr. *K. Mengel* (Hannover/Federal Republic of Germany):

On the question in regard to N-assimilation and ATP:
The binding of NH_4^+ to aspartic acid or glutamine acid needs ATP.

Prof. *H.J. Evans* (Corvallis/USA):

There may be an indirect role of K^+ in the process of N_2-fixation. N_2 fixation requires a reductant and a source of ATP. ATP for N_2 fixation must be supplied directly or indirectly by photophosphorylation and oxidative phosphorylation. There is definite evidence that both oxidative and photophosphorylation require K^+ or some similar cation.

Prof. *Y. Coïc* (Versailles/France):

1) *First reflection:* In the majority of the non-leguminous vegetal species the ammonium ions is not transported directly from the roots to the leaves, but is transformed before to amids in the roots.
2) *Question:* The bacteria have the property to accumulate phosphorus and energy in the form of polyphosphates minerals. Is K^+ necessary for the synthesis of these polyphosphates, as it is for that of ATP?

Dr. *H.J. Evans* (Corvallis/USA)

Since the synthesis of polyphosphates involves the synthesis of a high energy bond, one may presume that ATP or an equivalent amount of energy is needed.

Dr. *A. Kylin* (Stockholm/Sweden):

When it is stated that ATP is necessary for the formation of pyrophosphate, I have to make an objection. I do not know about the situation in *Rhizobium*, but it was shown by *H. Baltscheffsky* and his group that photo-induced transport of electrons in *Rhodospirillum* can give *either* pyrophosphate *or* ATP, with the choice between them made on the level of a high energy intermediate. Both pyrophosphate and ATP are biologically active in chromophores of *Rhodospirillum* as well as in chloroplasts of higher plants. In my own group, we have produced evidence for a similar situation in the microalga *Scenedesmus* as regards the determination of whether inorganic polyphosphates or ATP is to be produced by photophosphorylation.

Dr. *P.M. Riis* (Copenhagen/Denmark):

In one of the first slides Dr. *Evans* had listed some enzymes requiring K^+. The table also gave some values for concentrations of K^+. Did these values indicate the concentrations needed for maximum activity of the enzymes?
For the enzyme fructose 1.6-diphosphatase the table gave a value of 0.1 *M*, which is well above the highest value he gave for K^+-concentration in normal fresh leaves. Do you think that K^+-concentration normally limits the activity of an enzyme such as fructose-1.6-diphosphatase? I think, we can conclude from what Dr. *Evans* said, that this question concerns not only the particular enzyme mentioned but mainly other ones. It appears that K^+ has a general effect on conformation of practically all proteins and hence an allosteric effect on many enzymes. I believe this idea is in accordance with the results presented by Dr. *Hecht-Buchholz*. Dr. *Evans* mentioned that it has been shown for about 60 enzymes that they have a specific requirement for K^+. I wonder whether there are any enzymes, which are absolutely unaffected by K^+. Dr. *Evans* concluded that the concentration of K^+ required for maximum activity of many enzymes was of the same order as the concentration found in normal leaves. However, would it not be worthwile to emphasize that for some important enzymes the optimum K^+-concentration is higher than that found in normal fresh leaves. If this is significant, does it not mean we should look for means of increasing the cellular potassium level so that potassium is no longer a limiting factor?

Prof. Dr. *Dj.B. Jelenic* (Zemun-Belgrade/Yugoslavia):

1) In the Table 2 there are shown the data of the effects of N, K and Na on yields and % of soluble carbohydrates in the second cut of ryegrass. Have you conducted the experiment with the different levels of phosphorus in relation to N, K and Na?
2) Have you the results from your experiments on the effects of potassium and sodium on the protein content and its amino acids composition? (to compare with the data on free amino acids – Table 3)

Dr. *T.Z. Nowakowski* (Harpenden/England):

1) No, I have not investigated the effect of different levels of phosphorus on soluble carbohydrates.
In my experiment each pot received 1.0 g $Ca(H_2PO_4)_2.H_2O$ (246 mg P) to swanp out any effect of P.

2) Literature evidence suggests that mineral nutrition has very little effect on the quantitative distribution of individual amino acids in the protein hydrolysate, which is influenced more by genetic factors. Therefore, my main interest was concerned with various non-protein nitrogenous fractions. Nevertheless, the topic touched by you is a project of subsequent work.

Dr. *R. Scott Russell* (Wantage/England):

The clarity of Dr. ***Hecht-Buchholz's*** paper has left no need for questions, but this discussion would be incomplete without reference to the particular quality and value of the photomicrographs and electron-micrographs which she showed us. To my mind they provided an excellent example of one way in which modern experimental methods are increasing our ability to study, in detail, many aspects of the absorption and metabolic effects of potassium and other nutrients. This type of work holds promise of placing many of our vague present concepts on a firmer foundation.

Prof. Dr. *I. Arnon* (Rehovot/Israel):

1) Refer to experiment on effect of several cations on nitrate-reductase synthesis. K-deficient treatment gave a relatively steep increase in N-reductase formation after 3–4 hours. No indication that this was the peak of the graph. Has any investigation been made after 4 hours?
2) Have you considered the possibility of using one or more of the 60-odd enzyme activities in which K plays a vital role, for developing a diagnostic method for K-requirements of plants?

Prof. *H.J. Evans* (Corvallis/USA):

1) In our investigations of the effect of univalent cations on the inductive synthesis of nitrate reductase we purposely kept the induction period as short as possible in an effort to avoid complicating factors. We felt that initial rates of synthesis would provide the most reliable information. No investigations were made on induction of nitrate reductase for periods greater than 4 hours.
2) We have considered the feasibility of using a potassium activated enzyme as an indicator of potassium requirements and have concluded that too many complications are possible. One has to consider the efficiency of extraction of the enzymes from plant materials. Very seldom is it possible to quantitatively extract enzymes from plant tissues. Also it is difficult to perfect highly reproducible extraction procedures. Also since there are excellent assays for potassium contents of tissues, we feel that potassium analysis is the most reliable method for determining potassium requirements.

Dr. *A. Kylin* (Stockholm/Sweden):

In plants where there is no obvious vital need for sodium although sodium has a beneficial effect, one could imagine that the linked transport of Na and K enters the picture. A certain amount of Na to be extruded, might help to keep the vital K inside the cytoplasm. With too little Na present, K may enter the transport system from both sides of the membrane, that is, K would be circulated in the membrane, may be with the risk of being lost to the medium.

Prof. Dr. *K. Mengel* (Hannover/Germany):

If there is an outwardly directed Na pump in the root cells of higher plants the Na efflux should be affected by metabolic conditions. Efflux experiments we carried out with maize roots did not show an effect of metabolism upon the Na efflux.

Dr. *A. Kylin* (Stockholm/Sweden):

a) We have not tried maize, so the question is difficult to answer. With regard to its physiological behaviour, one can perhaps guess the situation may resemble the one in the (low salt, high proportion of potassium) line of sugar-beets; that is, a predominance of a transport system localized in the plasmalemma.
b) I think the status of our ideas is that of a reasonable working hypothesis, which covers a good number of experimental data from the literature. The hope is that, starting from the hypothesis, useful experiments can be devised so that new facts are recorded, which in turn may lead to modifications of the hypothesis.

Prof. Dr. *I. Arnon* (Rehovot/Israel);

Have you considered the possibility of using your results for developing a tool for plantbreeders for isolating saltresistant strains?

Dr. *A. Kylin* (Stockholm/Sweden);

Yes, I think that this approach would be a sound way to breed for salt resistance, although I cannot promise quick results.

Dr. *W. Dijkshoorn* (Wageningen/The Netherlands):

The experiment on *Scenedesmus* showed a loss of Na from the cells in terms of concentration in the cells.
Is it possible that after adding phosphate the cells grow so fast that the concentration falls as a result of dilution by growth with no fall in the total amount of Na in the cells?
Dr. *Kylin* said that growth was not so fast as to interfere in this way.

Dr. *A. Kylin* (Stockholm/Sweden):

Growth was no problem for our work with *Scenedesmus*. The experiments were done in a series of vessels with the same amounts of inoculate and the same amounts of medium. All cells in a vessel were harvested at the same time and circularly mounted for counts of their total contents of Na (^{22}Na was used in the experiments). Furthermore, the experimental time was too short to give appreciable growth; and some experiments were performed in darkness, where no growth at all can occur.

Prof. Dr. *K. Mengel* (Hannover/Federal Republic of Germany):

Is there sufficient evidence that in cells of higher plants an outwardly directed Na pump is existing?

Dr. *A. Kylin* (Stockholm/Sweden):

Rothstein (reference cited in my paper) once pointed out that sodium extrusion is necessary for naked cells, to prevent overhydration of the cytoplasm; but that the function could be less important in plants, where the mechanical pressure exerted by the cell wall may replace the need for sodium extrusion. This is one of the reasons why we started our work with plants known to be halophytes, where the need for well developed sodium extrusion must be greater than in glycophytes. Our general idea is that, once the system is known and defined, it may be possible to trace it also in more glycophytic plants; that is, plants exerted to less sodium stress.

One should, however, realize that there are plants where the protection against sodium occurs at the root level. For instance, the mangrove rhizophora does not allow sodium to pass the roots. No data on such plants are available, but there is an alternative biochemical possibility, which should be explored. Antibiotics of the valinomycin type represent carrier systems with a high grade of specificity, which makes the membranes permeable for potassium but not for sodium. It is, therefore, conceivable, that some plants may have developed very specific carriers to take different hydrophilic ions through the lipophilic parts of the membranes. Protection against sodium could then be obtained by a barrier of differential permeability. We know nothing about this, but the possibility should be born in mind.

Co-ordination Lecture for Session No. 1

Prof. Dr. D.B. JELENIC, Head of Department of Agricultural Chemistry and Plant Physiology of the Faculty of Agriculture, University of Belgrade, Yugoslavia; Member of the Scientific Board of the International Potash Institute.

The knowledge of the problems concerning biochemical and physiological functions of potassium in plants is very important, not only for the theoretical aspect of plant biochemistry, but also for the practical actions in intensification of agricultural production. Potassium is an essential element for all living organisms and the most abundant cation in the tissues of higher plants. There are numerous consequences of potassium ion deficiency in plant organism – (a) soluble carbohydrates and reducing sugars accumulation; (b) starch and glycogen syntheses are impaired; (c) amino acids accumulate and protein synthesis is blocked; (d) the utilization of respiratory substrates is retarded; (e) oxidative phosphorylation and photophosporylation rates are decreased etc., and it seems clear that potassium ion has a fundamental influence on a whole series of different metabolic processes in plant.

Detailed biochemical investigations may provide more definitive information on the potassium role concern:

- protein synthesis and enzyme activation
- carbohydrate metabolism and starch synthesis
- photosynthesis and respiration, etc.

Not all physiologists agree on the relative importance of the mechanism involving potassium. They are certainly very diversified and the same modes of action are cited in the main lecture presented by Prof. *Evans*, and in the communications presented by Dr. *Hecht-Buchholz*, Dr. *Nowakowski*, Prof. *Marschner* and Dr. *Kylin*.

Taking into account the subject matter of this Colloquium, the main lecture and the contributions at this Session concerning the 'Potassium in Biochemistry and Physiology of Plant', had a special significance.

In the main lecture Prof. *Evans* gives the essential scientific information on the fundamental role of potassium in plant organism. Potassium is a cofactor for a large number of enzymes that participate in several major metabolic processes. In their papers authors have reviewed the evidence for the necessity of potassium and other univalent cations for the syntheses of protein and starch, two metabolic processes of primary economic importance for food production by the agricultural industry. Of all univalent cations present in normal living tissues, potassium is the only cation that has the appropriate properties and that is present in cells in sufficient concentration to fulfill the univalent cation requirements of the great majority of those enzymes whose activities are dependent upon univalent cations.

In regard to the mechanism of potassium activation of enzymes Prof. *Evans* has concluded some facts: (a) the subunit structure of some enzymes is dependent upon the type of the present univalent cation and the capacity of some enzymes to bind a particular coenzyme is univalent cation dependent.

Special to say that (b) univalent cations such as potassium may behave as allosteric effectors; and (d) univalent cations may influence the conformation of some enzymes without causing big changes in physical structure.

In this session was not discussed the problem of the potassium influence on the photosynthesis. But, recent experiments have confirmed the universal need for potassium in the mechanisms of photosynthesis. Any shortage of potassium leads to a reduction of photosynthetic activity, a reduction that may or may not be reversible according to the severity of the deficiency. This effect will be the result of an alteration of protein metabolism and of the impaired functioning of a specific enzyme. Potassium will have a direct effect on the production of chlorophyll, on which photosynthesis directly depends.

In his communication, Dr. *Nowakowski* has studied the problem *'The effects of potassium and sodium on the carbohydrates and some protein metabolism in grass'*. Many observers have confirmed that protein metabolism is disturbed in plants inadequately provided with potassium. In potassium deficiency, there is an accumulation of aminoacids and amids in place of proteins. The soluble nitrogen compounds accumulate on account of an intensive hydrolysis of proteins, no longer counterbalanced by an equivalent amount of polymerisation as would be the case in a cell normally stocked with potassium. Dr. *Nowakowski* gives us a view of what we need for the approach of this problem.

Dr.*Ch. Hecht-Buchholz* has choiced to give us the results of her investigation with the aim to find specific changes in the fine structure of the cell after removal of potassium. Using electronmicroscopic techniques in her investigations.

Dr. *Hecht-Buchholz* finds that the removal of potassium caused a remarkable change in the fine structure of the proplastids, and the findings she has discussed in relation to the functions of potassium in carbohydrate and protein metabolism.
Prof. *Marschner* as well as Dr. *Kylin* and Dr. *Hansson* pointed out the problems of replacement potassium by sodium *(Marschner)* and the transport of sodium and potassium in plant *(Kylin* and *Hansson)*. The extent of replacement of potassium by sodium depends upon the plant species.
The main lecture and the communications have given us a very good contribution and very interesting statement of greatest value.

2nd Working Session — **Potassium in Biochemistry and Physiology of Plants**

Chairman of the Session: Prof. Dr. *K. Mengel*, Director, Agricultural Research Station Büntehof, Hannover/Federal Republic of Germany

The Uptake and Distribution of Potassium in Crop Plants

R. Scott Russell, D. Sc., Ph.D., F.I. Biol. and D.T. Clarkson, B. Sc., Ph. D., Agricultural Research Council Letcombe Laboratory, Wantage/England

The subject we have been invited to review, the uptake and distribution of potassium by crop plants, could be interpreted as embracing almost every aspect of the absorption and utilization of this element by the intact plant. A comprehensive discussion of the whole subject is thus impossible and it has seemed appropriate to direct attention mainly to aspects on which there has been progress in the recent past or on which the need for further information is particularly evident. On this basis, relationships between the structure and physiology of root systems and their ability to absorb potassium and translocate it to other parts of plants are an obvious starting point.

1. *Root structure and the absorption of potassium*

The total quantity of nutrients which intact root systems can absorb in solution culture or from the soil has been studied extensively. There is also much information on uptake by detached roots but it could be stated less than five years ago, 'only a very incomplete picture is still available of the relative extent to which ions enter or are translocated from different parts of the root system of any plant' *(Russell* and *Sanderson [38]);* the intervening years have added to our knowledge which is, however, still confined to very few species. None the less the agricultural relevance of identifying the parts of root systems where absorption and translocation mainly occurs is obvious. The older zones of root systems, which are relatively close to the soil surface, frequently experience very different environmental conditions from the younger tissues which develop at a greater depth. The significance of the contrasting distribution of root systems which can arise both from genetic and environmental causes can be judged only with knowledge of the performance of their component members. The academic interest of understanding how the successive processes of nutrient absorption and translocation change as roots mature could scarcely have been expressed more clearly than by *Prevot* and *Steward [35];* yet for nearly three decades little new information was forthcoming to supplement their preliminary findings. Experimental difficulties provide the explanation. The extent to which ions enter any one part of an intact root system, or are translocated from it, can be greatly influenced by the activity of other parts of the root system. Thus to obtain unequivocal information on the contribution of different root members to the nutrition of the intact plants, it is essential that nutrients which reach shoots from any one zone should be distinguished from those contributed simultaneously from elsewhere. This requirement could be fully satisfied only when radioactive tracers became available; previously it had

been necessary to have the recourse to such expedients as applying ions not normally encountered by the plant (e.g. bromide) and inferring the normal uptake pattern from the results obtained. This method was subject to obvious limitations but more recent workers have reason to admire the manner in which *Prevot* and *Steward* [*35*] defined subjects for subsequent study by this means.

1.1 *Experimental methods*

The size and complexity of root systems makes evident the magnitude of the task of mapping the pattern of uptake even in an approximate manner. A cereal root system, for example, may within two or three weeks of germination develop over 50 metres of root comprising upwards of a dozen seminal and nodal axes, the longest of which may exceed 0.5 metre, and perhaps 2000 laterals of variable length. If an intact root system has been exposed for short periods to nutrients labelled with radioactive tracers, it is now relatively simple by electronic scanning to compare the quantities retained by different root members *(Bowen* and *Rovira* [*3*]*)*. However, a complete understanding of the contribution of individual root members requires simultaneous measurements of translocation as well as uptake. Selected zones must therefore be provided with labelled nutrients while the remainder of the root system receives an unlabelled supply.

Gregory and *Woodford* [*20*] were among the pioneers in designing equipment for isolating different parts of root systems though they were interested largely in water uptake; *Brouwer* [*4*] used a basically similar design. However, to survey the entire root system this equipment had the serious disadvantage that only the apical unbranched zones of roots could be isolated for independent examination. The same limitation applied to simpler equipment developed by *Wiebe* and *Kramer* [*47*]; root apices were threaded through pairs of holes bored across the diameter of pliable tubing through which labelled nutrients were circulated after the holes had been sealed. A modification of this device (Figure 1) however enables it to be attached to any part of the root system *(Russell* and *Sanderson* [*38*]*)*. Holes wide enough to receive root segments are bored across the diameter of plastic tubing which is then cut through half its diameter so that when pressure is applied to the intact side the cut opens to receive the root; when the tube is suitably supported it is practicable, with sufficiently dexterous manipulation, to achieve an adequate seal with a number of non-toxic substances. Because of the considerable variability which occurs between seemingly comparable parts of different roots it is very important to make simultaneous measurements on comparable segments from different plants. It is also possible to measure water uptake by fitting micropotometers to the absorption cells *(Clarkson* and *Sanderson* [*9*]*)*.

So that observations can be made on zones of roots where lateral branches are relatively closely spaced, the absorption cells are usually made of narrow tubing (e.g. 3.5 mm diameter). Appreciable longitudinal movement occurs in the cortex but both retention in the treated segment and translocation from it can be reliably observed.

The work now to be described was conducted mainly with barley *(Hordeum vulgare* varieties Maris Badger and Midas) and in each experiment the entire root system was exposed to a uniform concentration of nutrients. Potassium was usually labelled with ^{42}K except in experiments with very dilute external solutions when ^{86}Rb was sometimes used as a tracer; there is evidence that the two elements move similarly in plant tissues.

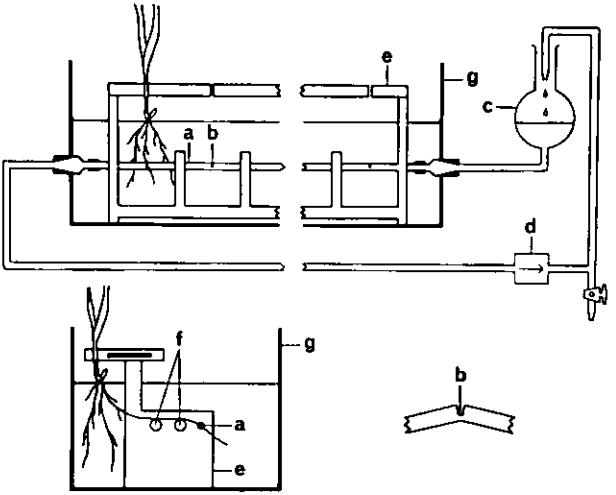

Figure 1. Apparatus for measuring the uptake of nutrients by different parts of intact root systems.
a. Absorption cell, made from polythene tubing, across the diameter of which roots are sealed.
b. Incisions in cell to receive roots; with pressure from below incision is opened for the entry of root (see inset).
c and d. Reservoir and peristaltic pump for circulating labelled solution in absorption cell.
e and f. Supports for absorption cell and plants.
g. Outer tank containing unlabelled nutrient solution.
Based on *Russell* and *Sanderson* [*38*]

1.2 *Root age and the absorption of potassium*

The young apical parts of roots in which the stele has lately differentiated have commonly been regarded as the main absorbing zone; the thin walls of the cells in this region would appear to provide little barrier to the movement of water and solutes and their high rate of respiration and abundant cytoplasmic contents, including numerous mitochondria *(Brown* and *Broadbent* [*6*]*)*, indicate high metabolic activity. In contrast the older root tissue appears less fitted for the radial transfer of ions. For example, 40 to 50 cm from the apex of the seminal root axes of barley the rate of respiration is lower (Table 1) the endodermis with its heavy tertiary thickening appears, under the light microscope, to form a virtually continuous sheath round the stele, there being few passage cells; moreover the outer cortical cells are frequently moribund and collapsed. None the less the absorption of

Table 1. Rates of oxygen consumption by excised segments of Barley root taken from various distances from the tip of seminal axes

Segment position (relative to root tip) (cm)	Qo_2 (μl O_2 g^{-1} [dry weight] min^{-1})
1– 2	91.5
7– 8	65.2
15–20	48.3
Basal (approx. 40)	30.8

Measurements on segments excised from 17 day old barley plants incubated at 20° without exogenous substrate.

potassium can occur along the entire length of the axis (Table 2); uptake by the older segments is usually smaller, but as large a fraction of that which has been absorbed reaches the stele and is translocated to shoots as from the apical zone.

Electron microscopy suggests an explanation of the ability of ions to traverse the heavily thickened endodermis in the basal regions of seminal root axes. Numerous pits occur in its inner tangential walls and plasmodesmata, passing through the pit floor, maintain protoplasmic continuity with the cells of the pericycle, thus providing a route for movement into the stele *(Clarkson et al. [8])*. The transfer of phosphate across these older root tissues, which is, in several respects, comparable with that of potassium has been studied in greater detail and calculations indicate that the number and cross-sectional area of the plasmodesmata should be sufficient to carry the observed flux of phosphate and also water into the stele.

No localized part of the cereal root system can thus be regarded as a unique absorbing zone; the entire root system possesses the ability both to absorb and translocate potassium provided that some cortical cells remain alive. However, experiments with phosphate suggest that when the entire cortex has collapsed transfer to the conducting tissue is likely to sink to a negligible level. The roots of dicotyledons have as yet been studied in less detail but experiments with *Cucurbita pepo (Murray* and *Clarkson [31])* suggest that the relationships between root structure and uptake in that species resemble those found in barley (Table 3).

Table 2. Uptake of potassium and translocation by segments (3.5 mm long) of intact root axes of Barley *(Hordeum vulgare)*

External concentration mM of potassium	1.0		0.1		0.1	
Type of axis	Seminal		Seminal		Nodal	
Distance from root apex (mm)	Uptake*	% translocated	Uptake*	% translocated	Uptake*	% translocated
10	11.5	40	1.69	48	1.10	40
20–40	11.7	64	2.32	61	5.29	55
60–80	12.8	71	—	—	1.86	71
150	—	—	0.94	48	2.54	57
>300	6.6	35	2.5	48	3.74	56

* *n* moles mm^{-3} day^{-1}

Growth conditions: Uptake solution, 0.1 or 1.0 m*M* KCl, 0.1 m*M* $CaCl_2$, 0.003 m*M* KH_2PO_4 pH 6.5
Temperature, 20 °C; relative humidity 65–75%; light intensity 2×10^4 lux; day length 16 hr.

Table 3. Uptake and translocation of potassium by segments (3.5 mm long) of intact primary roots of Marrow plants (*Cucurbita pepo* var. Greenbush) 10 days old

Distance of segment from root apex (mm)	Total uptake *n* moles mm^{-3} hr^{-1}	Percentage translocated
5	2.32 ± 0.35	54
10	1.97 ± 0.43	57
20	1.66 ± 0.22	67
150	2.38 ± 0.37	58
250	2.83 ± 0.48	54
300	1.26 ± 0.16	48
cir. 500	1.66 ± 0.31	49

Growth conditions: Uptake solution, 0.5 m*M* KCl, 0.15 m*M* Ca $(NO_3)_2$, 0.01 m*M* KH_2PO_4, pH 6.5.
Uptake period, 6 hr; temperature, 20 °C; relative humidity, 65–75%; light intensity 2×10^4 lux.
Based on *Murray* and *Clarkson [31]*

1.3 *Contribution of different root members to the potassium nutrition of the entire plant*

Observations on numerous segments of different types combined with information on the dimensions of the root system make it possible to estimate the contribution of the main components of the root system to the nutrition of the plant. This question has been studied more fully with phosphate and calcium but Table 4 shows a preliminary calculation for the uptake of potassium by barley plants 3—4 weeks old which had been grown with an abundant supply of nutrients. The important contribution of the laterals is evident. Over the entire root system there is no simple and consistent relationship between the uptake and translocation of potassium and the length, volume or surface area of the root tissue; relative to their dimensions the contribution of the thicker nodal axes is greater. However absorption is on average considerably more closely related to volume than to the other two parameters. It must be emphasised that this applies to root systems growing in well-stirred solutions so that all parts of the root system are exposed to a uniform external concentration. It is evident that under these circumstances absorption is not primarily controlled by the flux of ions per unit area of the root surface.

1.4 *Comparative uptake of potassium and other ions*

In discussions of interactions between ions in absorption it has usually been assumed that although their rates of transfer contrast, the distribution of absorbing power throughout the root system is comparable for all nutrients. Comparative studies of uptake of different ions by different parts of intact root systems now show that the situation is more complex. Potassium and calcium contrast in many respects in the extent to which they are absorbed and translocated by different types of tissue. In particular little calcium reaches the conducting tissues of the older parts of seminal root axes in which the tertiary endodermis is well developed *(Clarkson et al [10])*. The reason for the exclusion of calcium from this pathway, which is accessible to potassium, is not yet fully understood. In the younger parts of the root system the behaviour of the two ions differs less, but is not identical. The absorption of potassium and phosphate have also been compared; both traverse the older root members but the patterns of uptake of these two ions are again not identical. Sodium contrasts still more strikingly with potassium; for example appreciable movement of

Table 4. Estimated contribution of different types of root member to the absorption of potassium by the root systems of Barley plants 4 weeks old

	Entire root system	Percentage contribution of		
		Seminal axes	Nodal axes	Laterals
Dimension of root system*				
Length	5720 cm	8	7	85
Surface area	467 cm^2	12	18	70
Volume	3.8 cm^3	15	38	47
Absorption of potassium**				
Total uptake	9.4 μ moles day^{-1}	5	42	53
Translocated	4.3 μ moles day^{-1}	6	44	50

* From *Hackett [21]* ** Calculated from measurements of representative root segments
Growth conditions: Uptake solution, 0.1 m*M* KCl, 0.1 m*M* $CaCl_2$, 0.003 m*M* KH_2PO_4 pH 6.5.
Temperature, 20°C; relative humidity 65–75%; light intensity 2×10^4 lux; day length 16 hr.

sodium into the vascular tissues occurs in young portions of the roots of *Zea mays*, but with increasing distance from the root tip discrimination against the absorption of sodium, relative to potassium, increases markedly *(Shone et al. [41])*.

2. *Absorption by roots: an 'active' process*

Discussions of the uptake of ions by plants are frequently concerned primarily, if not entirely, with their entry into roots and subsequent radial transfer to the vascular tissue. This, however, is only one aspect of the subject and, as will be shown later in this review, the metabolic requirements of the shoot can on occasions exert a dominant influence on the quantity of potassium which is absorbed. None the less, it is appropriate to refer first to the general nature of the processes which occur in roots, though the extensive literature on this subject makes it unnecessary to provide more than a brief summary.

It is well established that although ions can enter by diffusion into the 'free space' external to the endodermis, continuing uptake is dependent upon metabolism. This is demonstrated by the influence of the rate of respiration and the supply of carbohydrates on absorption, as well as by the effects of respiratory inhibitors. Moreover, the concentration of ions in the vascular tissue can, in some circumstances, considerably exceed that in the outer media.

None the less, when the external salt concentration is high, nutrient uptake can be closely correlated with the rate of transpiration and this has sometimes encouraged the view that ions can also traverse roots passively by mass flow in water *(Hylmö [27])*. This view was, however, shown to be untenable many years ago by *Broyer* and *Hoagland* [7]. In plants of low salt status the absorption of potassium and bromide was virtually independent of the movement of water through the root; salt uptake varied with transpiration only when plants were of high salt status. These workers and many others since have concluded that the transfer of ions across plant roots was dependent on metabolism, but that the subsequent upward movement to the shoot could occur passively in the water stream. In plants of high salt status this latter process was considered to be rate limiting so that variations in transpiration affected the rate of the overall process. Investigations by *Russell* and *Shorrocks* [39] support this conclusion; when the rate of transpiration and the external nutrient supply are both low, the concentration of rubidium (and it may be presumed potassium) in the transpiration stream may be 30 or more times that in the ambient medium.

It can be concluded therefore that ions do not pass from the soil to the shoots of growing plants by mass flow but none the less it does not follow that all ions are necessarily *actively transported* in the strict sense of the term, that is to say that they are moved against a gradient of electrochemical potential, or 'uphill' thermodynamically. The active transport of one ion could cause an ion of opposing sign to move from a compartment of lower to higher concentration, thus maintaining the electrostatic balance. But the movement of this latter ion would, in strict terms, be passive as metabolic energy would not be directly expended on restraining it during transfer.

This subject can be investigated by comparing the concentration of an ion within a tissue with that which would be expected, on the basis of the Nernst equation, if it were in electrochemical equilibrium with the external medium for any observed value of the membrane potential. If the observed internal concentration is higher than that predicted, the ion has been moved actively, but if it is lower passive diffusion can be responsible. Such

calculations are strictly valid only when there is flux equilibrium, both the external concentration and that in the tissues remaining constant. This condition is rarely satisfied in growing roots but, when they and other plant organs are excised and kept in mineral salt solutions steady state conditions can be approximately attained. This procedure was used by *Higinbotham et al.* [*24*]. Their results with excised roots of *Avena sativa* in salt solutions containing 1 m*M* KCl are shown in Table 5. The predicted values are the equilibrium concentrations of the ions expected at the electrical potential difference which was observed across the plasmalemma, viz. −84 mV. The magnitude and arithmetic sign of the difference between the observed and predicted concentrations reflects the degree of departure from equilibrium and hence the direction of the passive driving forces of diffusion. Thus potassium entered the cells against an electrochemical gradient so that the maintenance of the steady state concentration of that ion in the roots was dependent on active transport; the results for calcium were, however, compatible with passive diffusion. Other evidence of active transport of potassium into roots has been provided by *Pitman* [*34*].

The concentration of potassium in the work illustrated in Table 5 was near to, or above, the maximum that plant roots would normally meet with in agricultural soils. However it is interesting, in connection with proposed carrier mechanisms, that *Higinbotham et al.* showed that a 10-fold increase in the external concentration of potassium reversed the direction of the diffusion gradient for that ion. In these circumstances the internal concentration was less than half the predicted equilibrium value, which suggests that when the external concentration is sufficiently high, uptake may be less directly dependent on metabolism. However, from the viewpoint of plant growth little interest attaches to the response of plants to salt concentrations much in excess of those they normally experience.

On the basis of kinetic analysis of relationships between external concentration and the absorption of potassium and other ions by detached roots it has been suggested that two different carrier mechanisms bring about absorption in roots, one being operative when the external concentration is low, the other when the external concentration is high *(Epstein* [*15*], *Laties* [*28*]*)*. It is unfortunate that these investigations were not carried out under rigidly sterile conditions as it is now well established that unless micro-organisms are rigorously excluded they can be responsible for a considerable part of the apparent uptake of potassium and rubidium by plant roots from solution *(Barber* [*1*]*)*. Figure 2 compares the absorption of rubidium in 24 hours from solutions containing from

Table 5. Comparison of observed steady-state concentrations of ions in excised roots of *Avena sativa* with those predicted if ions were in electrochemical equilibrium

Ion	Concentration in external medium (m*M*)	Steady-state concentration in plant (m*M*)		Direction of diffusion gradient
		Observed	Predicted*	
K	1.0	66	27	root → external medium
Ca	1.0	3	1400	external medium → root
H_2PO_4	1.0	17	0.04	root → external medium

* Predicted values calculated from the equation: $Cj^i = Cj^o \exp. \pm \frac{ZjFE}{RT}$

where E = potential difference across the membrane (−84 mV);
Cj^i and Cj^o = the concentrations of the ion j inside the cell and the outside solution;
Zj = algebraic valency of the ion j;
F = Faraday (constant) and other symbols have their usual meaning.
Based on *Higinbotham et al.* [*24*]

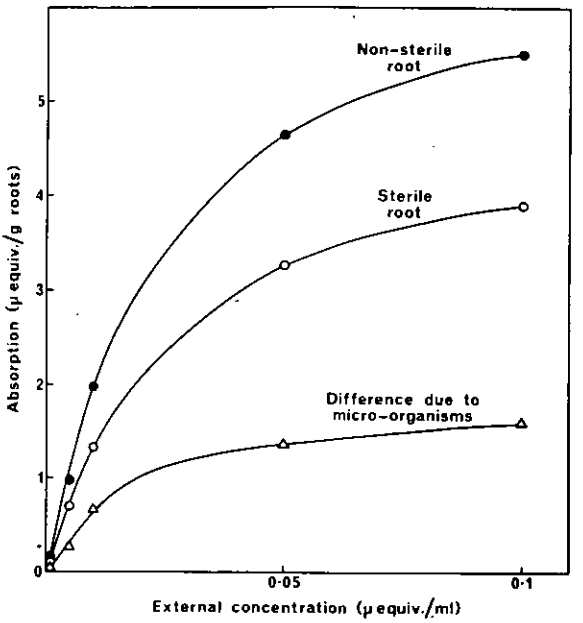

Figure 2. Effect of micro-organisms on the uptake of rubidium by detached barley roots from solutions of varying concentration of Rb Cl in the presence of 0.05 m*M* $CaCl_2$.
Based on *Barber* and *Frankenburg* [*1*]

0.01—0.1 meq rubidium l^{-1} by sterile and non-sterile detached roots of barley. Both sets of roots had been grown from sterile seed under rigidly sterile conditions but the non-sterile roots were subsequently infected through aeration with unfiltered air. Apart from the microbial infection the two sets of roots were thus identical so that differences between the quantities of rubidium they have absorbed shows the microbial contribution in the unsterile roots. It is thus evident that over the lower part of the concentration range illustrated in Figure 2, micro-organisms rather than plant physiological processes can be largely responsible for the apparent absorption by roots, though at higher concentrations their proportionate contribution is smaller. These findings show the desirability of re-examining carrier hypotheses under rigidly sterile conditions.

3. *Distribution and utilization by the whole plant*

Potassium is both the most abundant and the most mobile cation within plants and its internal translocation is strongly directed to points where active growth is occurring. It is essential in a wide range of osmotic and metabolic functions, some of which are highly specific for potassium. The close involvement of potassium in growth appears to exert a feed-back type of control over the rate of potassium absorption by roots.

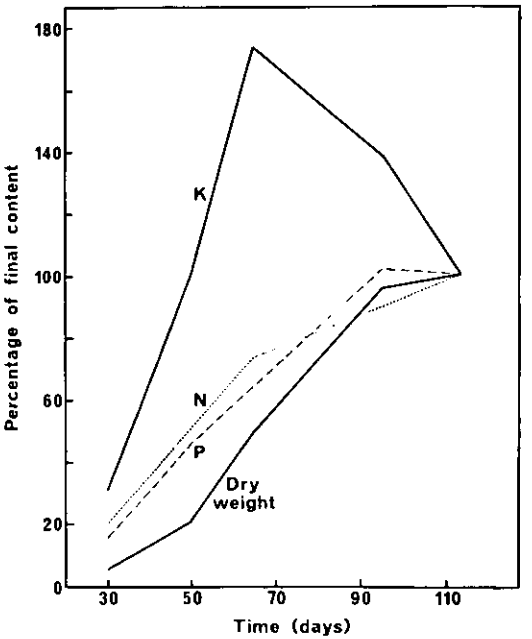

Figure 3. Changes with time in the nutrient content and dry weight of spring wheat under field conditions. Based on *Woodford* and *McCalla* [*49*]

3.1 *The translocation of potassium to meristematic zones*

After the initial absorption and radial transport to the vascular system, potassium, like other nutrients, moves towards shoots in the xylem, but unlike divalent ions, e.g. calcium, is not subject to restraint by ion-exchange reactions *(Bell* and *Biddulph* [*2*]*)*. Its movement is strongly directed to the meristematic zones of leaves and stems although movement into mature and even senescent leaves may occur. In leaves of these latter types the cessation of meristematic activity results in a net export of potassium out of the leaf to growing parts of the plant. Transport is presumably through the phloem which has been found in some species, e.g. *Salix*, to contain concentrations of potassium as high as 2% w/v *(Peal* and *Weatherley* [*33*]*)*.

Detailed information on the turnover of potassium in plants is surprisingly meagre but an interesting general illustration was provided by *Greenway* and *Pitman* [*18*] who applied ^{42}K at the third leaf stage to barley plants which had previously received the same concentration of potassium but in unlabelled form. Thus in the subsequent days potassium which had recently entered could be distinguished from that which had been absorbed before the ^{42}K was applied. Table 6 is based on their results. In the oldest leaf which was mature, but not senescent, the intake of potassium from the roots was broadly similar to that simultaneously lost by redistribution so that the potassium content of that leaf

Table 6. Turnover of potassium in leaves of Barley plants when the third leaf has emerged
Concentration of external solution: 2.5 m. equiv./l K^+ with a balanced supply of other nutrients

Tissue	Oldest leaf and coleoptile (L1)	Second leaf (L2)	Youngest leaf (L3)
Initial* dry weight (mg)	20	13	1.8
Initial potassium content (μ equiv.)	46	26	2.9
Intake (μ equiv. day^{-1}):			
From roots	1.9	2.7	2.0
By distribution from other tissues	−1.6	0.7	1.3

* At commencement of period when intake rates were measured
Based on *Greenway* and *Pitman [18]*

changed little. The greater part of the potassium redistributed from this leaf reached the youngest leaf (number 3) which was then expanding; it also received potassium from the root and thus its content increased rapidly. Leaf number 2 was intermediate. The quantitative uncertainty of such assessments was emphasized by the authors but the general pattern appears to be beyond doubt and probably represents the general situation in other plants. In addition to redistribution within the shoot system there is a considerable interchange of potassium between the shoot and the root system. Mature leaves and stems of a variety of species may be injected with solutions containing a label for potassium (either ^{42}K or ^{86}Rb) and sufficiently large amounts of radioactive tracer move to the roots within 24 hours for this to be used as a technique for demonstrating the quantity of roots in bulk soil samples *(Russell* and *Ellis* [*37*]*)*. Our own experiments have shown that potassium can enter the phloem of intact segments of roots and be translocated directly to root meristems, whereas calcium appears to be translocated only towards the shoot in the xylem. Segments of root near to the apex of roots direct the major portion of translocated potassium to the root tip, while potassium absorbed directly in the meristem is mainly retained there *(Steward* and *Koontz* [*43*]*)*.

3.2 *Growth and the internal demand for potassium*

Since the pattern of internal distribution is so strongly determined by meristematic activity it follows that, at stages of the development of a plant where the number of active meristems is greatest, the potassium 'requirement' will also be greatest. The early stages of growth of a cereal plant when all or most of the leaves are still expanding is likely to be a period when an adequate supply of potassium from the soil is most critical; when leaves mature redistribution of previously absorbed potassium to the growing regions may partly offset limitations imposed by external nutrient supply. *Gregory* and *Richards* [*19*] found that barley plants were most likely to develop potassium deficiency symptoms with concomitant decreases in carbon assimilation when the fifth and sixth leaves on the main axis were expanding. Subsequently, however, carbon assimilation and growth returned to normal levels, and potassium deficiency symptoms diminished when the peak period of vegetative growth was passed and the development of the ear began. In addition to internal redistribution of potassium from mature to growing parts of the plant there is evidence that as the internal demand created by the meristems diminishes there is a net loss of potassium from the plant, for example *Woodford* and *McCalla* [*49*] found that at maturity the total potassium content of barley plants was only 60% of that at midseason (Figure 3).

It seems probable that most of the potassium loss will occur by leaching from ageing leaves *(Tukey [45])*, although there are some reports of the outward movement of potassium from mature roots *(Helder [23]);* net losses from actively growing roots are not usually observed *(Greenway* and *Pitman [18])*. The stage of growth can thus greatly influence the 'requirement' for potassium and changes in internal demand may consequently affect the rate at which it is absorbed by roots.

3.3 *Role of potassium in growth and metabolism*

Although it is clearly established that potassium is 'required' by growing tissues, many biochemical aspects of cellular metabolism remain poorly understood. Thus the reasons why potassium is an essential element in growth cannot yet be fully explained. Its more general function as a major cation maintaining the osmotic pressure of cells is easier to appreciate. The increase of cellular volume by mitosis and expansion requires a net increase in the salt content of the plant so that turgor of the cells is maintained. This is at least a partial definition of what is meant when growing tissues are described as 'sinks' for potassium and other ions.

There is evidence that potassium is rather specifically involved in osmo-regulation in certain plant cells and that its role cannot be taken over by related alkali cations. Thus the well known 'sleep' movements of leaves of acaciod plants which involve the closure of leaflets in darkness are probably due to relative changes in turgor pressure in the cells of the pulvinules which result from a net transport of potassium from cells in the ventral to those at the dorsal surfaces of the pulvinules *(Satter et al. [40])*. These results are particularly interesting since these leaflet-closure responses can be controlled by the phytochrome system and suggests an interaction between the permeability of membranes to potassium and photomorphogenetic responses.

Another interaction between light and the concentration of potassium in cells has been found in the opening and closure of stomata. It has been shown that light-induced opening of stomata in isolated strips of epidermis from *Vicia faba* is specifically dependent on the presence of potassium in the outer medium and that the degree of opening depends on the non-exchangeable absorption of potassium which increases the osmolarity of the sap in the guard cells *(Fischer [16])*. Sodium cannot be substituted for potassium *(Fischer* and *Hsiao [17])*. The specific involvement of potassium has been further demonstrated by *Thomas [44]* who showed that there was no specificity for the accompanying anion in the stomatal opening of epidermal strips from *Nicotina tobaccum. Thomas* also showed that the opening response was inhibited by 10^{-6}M ouabain which is known to inhibit adenosine triphosphatase pumping systems which transport potassium in other plant and animal cells *(Raven [36], Whittam [48])*.

A second role for potassium is that of a universal carrier of positive charges. In the course of metabolism many macromolecules synthesised within cells possess excess fixed negative charge. Cations become associated with these sites by electrostatic binding. The specificity of the binding site for potassium may be influenced by the interactions of the site and other groups in its immediate chemical environment *(Ling [29])*. As potassium is an abundant and mobile cation it seems probable that it can neutralize a high proportion of these fixed negative charges. It seems unlikely, however, from activity measurements in cytoplasm using ion-specific microelectrodes that more than a minor portion of potassium can be sequestered in this way in algal cells *(Vorobiev [46], Spanswick [42])*.

The elucidation of biochemical processes in which potassium directly participates is

rendered difficult not only because it is not a constituent of any stable organic compound but also because the first detectable effect of its deficiency is frequently a general retardation of growth. Thus an extensive study of potassium deficiency in *Zea mays* by *Hsiao et al.* [*25, 26*] showed that major effects on nucleic acid metabolism and protein synthesis – for example an increase in the ratio of amino acids to protein – could be detected only *after* the rate of growth had declined. Moreover, when potassium was provided to the deficient plants, the normal ratio of amino acids to protein was not restored until normal growth had been resumed. The excessive accumulation of amines and increased respiration in potassium-deficient barley plants was noted some years ago *(Coleman* and *Richards* [*11*], *Hackett et al.* [*22*], and is indicative of a general breakdown of control mechanisms. Although it has been established in cell-free systems that potassium is essential in protein synthesis at the stage when amino acids are deposited at the ribosomes from transfer RNA *(Lubin* [*30*]*)*, the foregoing results seem to indicate that the disruption of protein synthesis is a consequence rather than a cause of retarded growth in potassium-deficient plants. This is clearly a subject which merits more critical and intensive biochemical study.

4. *Conclusions: The control of potassium nutrition in intact plants*

Any generalization on the overall manner in which the potassium economy of plants is controlled must have an unsure foundation until the detailed nature of its participation in metabolism is more fully understood. Some useful purpose may, however, be served by considering the extent to which the potassium content of plants is determined, in different circumstances, by the two main groups of factors which have been indicated in the preceding discussion, namely those which control its transfer across roots to the vascular stele and the demand created by metabolic processes.

When the external supply of potassium is sufficiently low to limit plant growth there can be little doubt that the performance of the root system exerts the dominant control. The external concentration then limits the rate of absorption by any one part of the root system and the total uptake of the plant varies with the extent of root development. It may be noted also that when potassium is deficient, root growth, and especially the development of laterals, may be markedly retarded *(Hackett* [*21*]*)*, a situation readily compatible with the general effects of potassium deficiency on metabolism to which reference has already been made.

In modern agriculture, however, the use of fertilizers is increasingly removing restrictions on plant growth caused by nutrient deficiency. In the words of *Cooke* [*12*] 'fertilizers eliminate one natural limitation of crop growth – the supply from soil'. If, in addition to containing an adequate concentration of nutrients, the rooting medium is favourable for root growth and adequately supplied with water, nutrient uptake is no longer determined solely by the extent of root development. This is clearly indicated by the work of *Brouwer* and *Kleinendorst* [*5*] who have shown that under these conditions the removal of part of the root system has little influence on the nutrient supply which plants receive, absorption by the remaining part of the root system being increased. The ability of roots to modify their rate of absorption in response to the demands of the plant is also illustrated by other evidence. For example, *Drew* and *Nye* [*14*] showed that the absorption of potassium by parts of the intact root system of young rye grass plants *(Lolium perenne)* can be some five times greater when the rest of the root system is growing in a nutrient-free sand as opposed to soil of the same nutrient status. Such effects can occur within a few days, that is to say

before root growth is visibly affected. This is but the first stage in the response of root systems to a variable nutrient supply. Over a hundred years ago *Nobbe* [*32*] recognized that roots eventually proliferate in zones where nutrients are more abundant. More recent work shows that this effect is particularly marked when only a small part of the rooting zone is in a favourable nutrient environment; lateral development can then considerably exceed that which would occur if the same concentrations of nutrients were available to the entire root system *(Drew* and *Ashley* [*13*]*)*. These complex and varied responses of root systems show that neither the growth of roots nor their ability to absorb nutrients is determined solely by the local environment to which they are exposed. The enhanced absorption by part of the root system when the remainder has been removed might be explained on the basis that an increased supply of substrates is available from the shoot, but a more subtle explanation is necessary to account for the localized response of parts of root system to variations in the external nutrient supply. Such observations are interpretable in no other way than that nutrient requirement of the plant can influence the transport of ions across the root/soil interface and also the movement of metabolites to sites where subsequent root growth occurs. This situation is not unique for potassium – it occurs equally with other nutrients. As yet, however, we are able only to recognize that diverse metabolic processes in the intact plant are closely co-ordinate – no detailed explanation can be possible without much fuller understanding of growth control mechanisms, both in roots and shoots. These are perhaps the most intriguing of all aspects of plant physiology – they are perhaps also the most difficult to study.

Bibliography

1. *Barber, D.A.*, and *Frankenburg, U.C.*: The contribution of micro-organisms to the apparent absorption of ions by roots grown under non-sterile conditions. New Phytol. *80*, pp. 1027–1034 (1971).
2. *Bell, C.W.*, and *Biddulph, O.*: Translocation of calcium. Exchange versus mass flow. Pl. Physiol., Lancaster *38*, pp. 610–614 (1963).
3. *Bowen, G.D.*, and *Rovira, A.D.*: The influence of micro-organisms on growth and metabolism of plant roots. In Root Growth (Edited by W.J. Whittington), pp. 170–199, Butterworths, London 1969.
4. *Brouwer, R.*: Water absorption by the roots of *Vicia faba* at various transpiration strengths. I, II. Proc. Kon. Ned. Akad. Wet *C56*, pp. 106–115, 129–136 (1953).
5. *Brouwer, R.*, and *Kleinendorst, A.*: Effect of root excision on growth phenomena in perennial ryegrass. Jaarb. Inst. biol. scheik. Onderz. Landb. Gewass. *1965*, pp. 11–20 (1965).
6. *Brown, R.*, and *Broadbent, D.*: The development of cells in the growing zones of the root. J. exp. Bot. *1*, pp. 249–263 (1951).
7. *Broyer, T.C.*, and *Hoagland, D.R.*: Metabolic activities of roots and their bearing on the relation of upward movement of salts and water in plants. Am. J. Bot. *30*, pp. 261–273 (1943).
8. *Clarkson, D.T., Robards, A.W.*, and *Sanderson, J.*: The tertiary endodermis in barley roots: fine structure in relation to radial transport of ions and water. Planta *96*, pp. 292–305 (1971).
9. *Clarkson, D.T.*, and *Sanderson, J.*: Relationship between the anatomy of cereal roots and the absorption of water and nutrients. Agricultural Research Council Letcombe Laboratory Ann. Rep. 1970, pp. 16–25 (1971).
10. *Clarkson, D.T., Sanderson, J.*, and *Russell, R.S.*: Ion uptake and root age. Nature, Lond. *220*, pp. 805–806 (1968).
11. *Coleman, R.G.*, and *Richards, F.J.*: Physiological studies in plant nutrition. XVIII. Some aspects of nitrogen metabolism in barley and other plants in relation to potassium deficiency. Ann. Bot. N.S. *20*, pp. 393–409 (1956).
12. *Cooke, G.W.*: Using chemicals to increase soil productivity. Society of Chemical Industry Symposium, Lancaster, July 1969, pp. 45–54, 1970.
13. *Drew, M.C.*, and *Ashley, T.W.*: Effect of variations in the supply of nitrate on root development. Agricultural Research Council Letcombe Laboratory Annual Report 1970, pp. 7–8, 1971.
14. *Drew, M.C.*, and *Nye, P.H.*: The supply of nutrient ions by diffusion to plant roots in soil. II. The effect of root hairs on the uptake of potassium by roots of rye grass *(Lolium multiflorum)*. Pl. Soil *31*, pp. 407–424 (1969).
15. *Epstein, E.*: Dual pattern of ion absorption by plant cells and by plants. Nature, Lond. *212*, pp. 1324–1327 (1966).

16. *Fischer, R.A.:* Stomatal opening: role of potassium uptake by guard cells. Science *160*, pp. 784–785 (1968).
17. *Fischer, R.A.*, and *Hsiao, T.C.:* Stomatal opening in isolated epidermal strips of *Vicia faba*. II. Responses to potassium chloride concentration and the role of potassium absorption. Pl. Physiol., Lancaster *43*, pp. 1953–1958 (1968).
18. *Greenway, H.*, and *Pitman, M.G.:* Potassium retranslocation in seedlings of *Hordeum vulgare*. Aust. J. biol. Sci. *18*, pp. 235–247 (1965).
19. *Gregory, F.G., Richards, F.J.:* Physiological studies in plant nutrition. 1. The effect of manurial deficiency on the respiration and assimilation rate in barley. Ann. Bot. *43*, pp. 119–161 (1929).
20. *Gregory, F.G.*, and *Woodford, H.K.:* An apparatus for the study of the oxygen, salt and water uptake of various zones of the root, with some preliminary results with *Vicia faba*. Ann. Bot. N.S. *3*, pp. 147–154 (1939).
21. *Hackett, C.:* A study of the root system of barley. 1. Effects of nutrition on two varieties. New Phytol. *67*, pp. 287–300 (1968).
22. *Hackett, C., Sinclair, C.*, and *Richards, F.J.:* Balance between potassium and phosphorus in nutrition of barley. I. The influence on amine content. Ann. Bot. N.S. *29*, pp. 331–345 (1965).
23. *Helder, R.J.:* Analysis of the process of anion uptake of intact maize plants. Acta bot. neerl. *1*, pp. 361–434 (1952).
24. *Higinbotham, N., Etherton, B.*, and *Foster, R.J.:* Mineral ion contents and cell transmembrane electropotentials of pea and oat seedling tissue. Pl. Physiol., Lancaster *42*, pp. 37–46 (1967).
25. *Hsiao, T.C., Hageman, R.H.*, and *Tyner, E.H.:* Effects of potassium nutrition on ribonucleic acid and ribonuclease in *Zea mays* L. Pl. Physiol., Lancaster *43*, pp. 1941–1946 (1968).
26. *Hsiao, T.C., Hageman, R.H.*, and *Tyner, E.H.:* Effects of potassium nutrition on protein and total free amino acids in *Zea mays*. Crop Sci. *10*, pp. 78–82 (1970).
27. *Hylmö, B.:* Passive components in the ion absorption of the plant. II. The zonal water flow, ion passage, and pore size in roots of *Vicia faba*. Physiologia Pl. *11*, pp. 382–400 (1958).
28. *Laties, G.G.:* Dual mechanisms of salt uptake in relation to compartmentation and long distance transport. A. Rev. Pl. Physiol. *20*, pp. 89–116 (1969).
29. *Ling, G.N.:* A Physical Theory of the Living State: the Association-Induction Hypothesis. Chapt. 4, Blaisdell, New York 1962.
30. *Lubin, M.:* Intracellular potassium and the control of protein synthesis. Fedn. Proc. Fedn. Am. Socs. exp. Biol. *23*, pp. 994–1001 (1964).
31. *Murray, R.S.H.*, and *Clarkson, D.T.:* Absorption and translocation of ions by the root system of marrow (*Cucurbita pepo*, c.v. Greenbush). (In preparation).
32. *Nobbe, F.:* Über die feinere Verästelung der Pflanzenwurzeln. Landw. VersSta. *4*, pp. 212–224 (1862).
33. *Peel, A.J.*, and *Weatherley, P.E.:* Composition of sieve-tube sap. Nature, Lond. *184*, pp. 1955–1956 (1959).
34. *Pitman, M.G.:* Sodium and potassium uptake by seedlings of *Hordeum vulgare*. Aust. J. biol. Sci. *18*, pp. 10–24 (1965).
35. *Prevot, P.*, and *Steward, F.C.:* Salient features of the root system relative to the problem of salt absorption. Pl. Physiol., Lancaster *11*, pp. 509–534 (1936).
36. *Raven, J.A.:* Light stimulation of active transport in *Hydrodictyon africanum*. J. gen. Physiol. *50*, pp. 1627–1640 (1967).
37. *Russell, R.S.*, and *Ellis, F.B.:* Estimation of the distribution of plant roots in soil. Nature, Lond. *217*, pp. 582–583 (1968).
38. *Russell, R.S.*, and *Sanderson, J.:* Nutrient uptake by different parts of the intact roots of plants. J. exp. Bot. *18*, pp. 491–508 (1967).
39. *Russell, R.S.*, and *Shorrocks, V.M.:* The relationship between transpiration and the absorption of inorganic ions by intact plants. J. exp. Bot. *10*, pp. 301–316 (1959).
40. *Satter, R.L., Marinoff, P.*, and *Galston, A.:* Phytochrome controlled nyctinasty in *Albizzia julibrissin*. II. Potassium flux as a basis of leaflet movement. Am. J. Bot. *57*, pp. 916–926 (1970).
41. *Shone, M.G.T., Clarkson, D.T.*, and *Sanderson, J.:* The absorption and translocation of sodium by maize seedlings. Planta *86*, pp. 301–314 (1969).
42. *Spanswick, R.M.:* Measurements of potassium ion activity in the cytoplasm of the Characeae as a test of the sorption theory. Nature, Lond. *218*, p. 357 (1968).
43. *Steward, K.K.*, and *Koontz, H.V.:* Transport of rubidium absorbed by the apex of the bean root. Pl. Physiol., Lancaster *43*, pp. 583–588 (1968).
44. *Thomas, D.A.:* The regulation of stomatal aperture in tobacco leaf epidermal strips. II. The effect of ouabain. Aust. J. biol. Sci. *23*, pp. 981–989 (1970).
45. *Tukey, H.B.:* The leaching of substances from plants. A. Rev. Pl. Physiol. *21*, pp. 305–324 (1970).
46. *Vorobiev, L.N.:* Potassium ion activity in the cytoplasm and vacuole of cells of *Chara* and *Griffithsia*. Nature, Lond. *216*, pp. 1325–1327 (1967).
47. *Wiebe, H.H.*, and *Kramer, P.J.:* Translocation of radioactive isotopes from various regions of roots of barley seedlings. Pl. Physiol., Lancaster *29*, pp. 342–348 (1954).
48. *Whittam, R.:* The asymmetric stimulation of a membrane adenosine triphosphate in relation to active cation transport. Biochem. J. *84*, pp. 110–118 (1962).
49. *Woodford, E.K.*, and *McCalla, A.G.:* The absorption of nutrients by two varieties of wheat grown on the black and gray soils of Alberta. Can. J. Res., C *14*, pp. 245–266 (1936).

The Equilibrium between Potassium and other Cations in the Organs of Higher Plants

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Summary

Mineral cation composition of plant organs depends essentially upon processes of absorption, transport and accumulation, which themselves depend upon the type of plant under consideration and upon the environment. We emphasize the important influence of NO_3^- metabolism on these processes especially the great influence of the site of nitrate reduction (roots or leaves) on the cation content and composition of leaves and the development of these characteristics as influenced by various factors. The high mobility of potassium accompanying amino acids and soluble glucides explains the composition of organs to which these substances are supplied and it is also thought that potassium can normally perform several migration cycles, more especially in certain types of plant. The particular cationic composition of certain organs (stem nodes of cereals) demands attention as well as the accumulation of sodium in the aerial parts of some species or in the roots of others.

1. *Introduction*

Cation composition, specifically in K, Na, Ca and Mg, of plant species and organs depends upon the processes of absorption, transport and accumulation. All these processes are dependent upon species under consideration, in other words genetic factors, on the one hand, and composition of the growing medium on the other. Among environmental factors mineral nutrition is particularly important for the agronomist, in his aim to increase yield, that is to say the harvest of organic material. The comparative accumulation of a mineral element relative to increase in organic matter determines the content of that mineral on a dry matter basis.

Mineral cations are associated with mineral anions in the nutrient medium. Some mineral anions are subject to transformations in the plant of such a kind that the balance between anions and cations is modified as a result. It must therefore be conceived that transport and accumulation of ions depend very much upon the metabolism of particular anions. For example the Cl^- ion (not metabolised) and NO_3^- play very different roles in these processes. The nitrogenous ions, expressed on an equivalent basis, are absorbed and metabolized in very large quantity.

If the metabolism of ions has an important influence on absorption, transport and accumulation of cations, NO_3^- would be expected to have the greatest effect.

In accordance with the policy of this colloquium we shall not present a complete or extensive survey but consider only those experimental results which will allow us to give an explanation of the physiological processes concerned.

2. *Cationic composition of leaves.*

2.1 *Differences between species.*

The absorption of cations varies greatly according to species and is expressed more particularly in great differences in cationic composition of the leaves. Since we know that the type of nitrogen supply, ammonium or nitrate, is concerned in determining leaf composition, we have compared two plants which show typical differences in cationic composition of the leaf, viz. maize and tomato. These were both grown with N supplied either exclusively as ammonium or as nitrate. The two dropping nutrient solutions were made up in such a way that the concentration of each anion ($PO_4H_2^-$, $SO_4^=$, Cl^-) was the same, the concentrations of NH_4^+ and NO_3^- were equivalent and that the concentration of each cation was twice as high in NO_3^- as in NH_4^+ nutrition. Proportionality between cations in the nutrient solution was thus conserved, an essential condition in studies of variation in equilibria between cations in the plant.

The two following conclusions can be drawn from Table 1:

1) While the leaves of the two species have roughly similar cation content and composition under NH_4^+ nutrition there are large differences under NO_3^- nutrition.
2) While there is little difference in mineral content and composition of maize leaves between the two types of N — nutrition, great changes appear in tomato leaves.

Corresponding with this difference in content of mineral cations between maize and tomato there is a considerable and parallel difference in content of total organic acids (free and combined). We know that the amount of organic acid in the leaf is evidence for the amount of nitrate reduced there *(Arnon [1], Coïc et al. [4], Coïc [7], Coïc et al. [11], Coïc et al. [12])*.

A feature of the difference in behaviour between maize and tomato under nitrate nutrition is the difference in content of organic acid anions in the leaves of the two plants. A high proportion of nitrate taken up by tomato is reduced and metabolised in the leaf (thus losing its anionic property) while in maize a large proportion of nitrate has been metabolised in the root.

The nitrate ion is transported from root to leaf with an equivalent amount of cations and there is no specificity for particular cations, their proportions depending mainly upon the cationic composition of the nutrient supply to the plant. When nitrate is reduced in the leaf it is replaced by organic acid anions and those cations which are not fixed by cellular structures (particularly the walls) accumulate in the vacuole. When nitrate is metabolised in the root electrostatic equilibrium is achieved by compensatory absorption of further mineral anions, in particular of nitrate, that is to say absorption of anions is greater than that of cations thus causing *physiological alkalinisation (Coïc et al. [10])*. When nitrate is transformed into amino- or amido-acid it is transported with a lesser quantity of cations than is the case with NO_3^-. On the other hand there is some selectivity among cations accompanying this organic nitrogen and potassium is preferred in comparison to magnesium and more particularly to calcium.

We attribute, therefore, the difference in cation content between the leaves of maize and tomato to the difference in capability of the roots to metabolize nitrate: when NO_3^- absorption by the two plants is equal, maize metabolises a much greater proportion of NO_3^- in the roots than does tomato.

But why are divalent cations accumulated in preference to potassium in the tomato leaf?

The accumulation of an element in an organ reflects the balance between incoming and

Table 1. Coïc et al. [4]

meq/100 g fresh weight	Maize (blade)		Tomato (leaf)			
	NO_3^-	NH_4^+	NO_3^-		NH_4^+	
			young leaf	old leaf	young leaf	old leaf
Total N	45	49	55.1	31.1	75.6	50.2
Total P	7.5	12.4	8.2	5.0	14.8	15.2
K	13.1	13.1	11.5	10.8	8.2	8.3
Ca	6.2	4.0	20.6	33.0	2.9	5.3
Mg	5.2	3.3	7.9	11.4	1.8	3.9
Total cations	24.5	20.4	40.0	55.2	12.9	17.5
Organic acids (free and combined).	12.4	3.6	20.8	32.8	0.7	0.5

outgoing of that element. The very high content of calcium and moderately high content of magnesium in the tomato leaf is not obviously due to selection of Ca^{++} and Mg^{++} by mineral anions (particularly NO_3^- because it is the most abundant) in transport to the leaf. It is caused by transport from the leaf of K^+ bound with organic materials synthesized in the leaf.

Leaves of various species also differ greatly in accumulation of sodium.

2.2 *Comparison of leaves of varying age (Coïc [7]).*

With ageing, Mg and even more Ca assume greater importance among the cations. The leaf has a tendency to accumulate cations continuously (cation content of dry matter depending upon relative accumulation of cations in proportion to that of dry matter) but relative enrichment in calcium and magnesium in comparison with potassium is due to the fact that as the leaf ages it supplies more and more organic nutrient to other parts of the plant, particularly to storage organs. These migrating organic substances are mainly accompanied by potassium. If the storage organs cannot accumulate potassium the latter may be excreted, as happens, for instance, in cereals at the end of vegetative growth.

All this emphasizes the great mobility of potassium within the plant and we think that potassium may serve this purpose several times, that is to say for several cycles in the transport of substances.

On the other hand it is quite clear that in plants of the maize type the sum of cations in dry matter and cationic composition shows less variation with increasing age than is the case with plants of the tomato type, where accumulation of Ca and Mg increases progressively with increasing age. In effect, the accumulation of cations, and more particularly of Ca and Mg in the leaf depends entirely (as does the accumulation of organic acids) on the amount of nitrate which is metabolised there. When the plant metabolises a high proportion of the absorbed nitrate in the leaf, the difference in the amount of nitrate which is metabolised between young and old leaves is more important and results in a considerable difference in cationic composition $\frac{Ca + Mg}{K}$ *(Coïc et al. [11], Coïc et al. [12], Coïc et al. [13]).*

In plants whose leaves can accumulate sodium one finds that this element behaves as Ca and Mg and not as K as is shown in Table 2 relating to endive.

Table 2. Endive *(Cichorium Intybus)* – 1st year (meq per 100 g d.m.)

	Sampled 6th October		
	Young leaves	Adult leaves	Old leaves
K	98	70.5	46
Na	39	67	88
Ca	34.5	83.5	141.5
Mg	27	38	64

2.3 *Influence of various factors.*

a) Type of N nutrition

We have seen the effect of nitrate versus ammonium nutrition on cation balance in the leaf. The magnitude of variation is particularly marked in plants where nitrate metabolism in the leaf is normally an important process.

b) Effect of nitrate deficiency

If one suppresses nitrate supply to the tobacco plant (a type metabolizing much NO_3 in the leaf) cation content of the leaf is much reduced (−N or −N−N compared with +N or +N+N) and increases again considerably when NO_3 supply is restored (−N+N).

Table 3. (Coïc et al. [11]) Cation content (meq per 100 g d.m.)

		Young leaves	Adult leaves	Old leaves	Very old leaves	(S+P+V)
1st Harvest						
Plants −N	K	75.2	74.4	89.2	113.5	197.0
	Na	0.9	1.7	3.5	5.6	1.3
	Ca	33.0	36.5	68.0	159.5	20.5
	Mg	51.7	50.0	103.5	207.0	39.2
	Total	160.8	162.6	264.2	485.6	258.0
Plants +N	K	104.5	131.5	145.0	149.5	192.0
	Na	0.9	2.2	2.6	3.5	1.3
	Ca	62.0	83.0	117.5	170.5	19.5
	Mg	72.5	95.0	151.8	207.0	36.7
	Total	239.9	311.7	416.9	530.5	249.5
2nd Harvest						
Plants −N−N	K	60.5	60.0	86.2	115.0	119.0
	Na	0.9	2.2	4.8	7.0	0.9
	Ca	42.0	40.5	86.0	183.0	14.5
	Mg	46.7	48.3	119.0	242.0	25.8
	Total	150.1	151.0	296.0	547.0	160.2
Plants +N+N	K	106.0	122.0	129.0	129.0	192.5
	Na	1.3	2.6	2.6	4.8	1.3
	Ca	81.5	111.0	138.0	195.0	27.0
	Mg	85.0	113.0	166.0	222.0	42.5
	Total	273.8	348.6	435.6	550.8	263.3
Plants −N+N	K	113.0	121.0	137.0	137.0	144.0
	Na	1.3	2.6	3.5	5.6	1.3
	Ca	83.0	91.0	119.0	176.0	19.5
	Mg	88.5	107.8	147.0	218.0	36.0
	Total	285.8	322.4	406.5	536.6	200.8

S+P+V = Stems+Petioles+Veins

Absorption of the three cations, K, Ca and Mg is not lowered equally and it is K which varies most. The aerial part of the plant loses K while gaining a little Ca and maintaining Mg nearly constant.

While the old leaves only show a slight loss of potassium, the stem, petioles and main veins, very rich in K, lose an appreciable quantity of this element, the resumption of NO_3^- supply restores the situation favouring a gain of potassium by the leaves, even the old leaves which normally (+N +N) do not gain K during this period; but the stem, which is not yet fully supplied again with nitrates recovers its K content more slowly.

Table 4. (Coïc et al. [11]) Gains in cations (meq per plant)

		Young leaves (Ly)	Adult leaves (La)	Old leaves (Lo)	Ly + La + Lo	Very old leaves	Total lamina	S + P + V	Total
−N−N	K	4.3	−0.1	−2.3	1.9	−1.9	0	−19.8	−19.8
	Na	0.1	0.2	0.1	0.4	−0.1	0.3	− 0.1	0.2
	Ca	4.5	2.2	1.2	7.9	−2.5	5.4	− 0.6	4.8
	Mg	3.8	1.6	0.2	5.6	−3.3	2.3	− 2.5	− 0.2
	Total	12.7	3.9	−0.8	15.8	−7.8	8.0	−23.0	−15
+N+N	K	28.1	5.7	−1.0	32.8	−3.1	29.7	105.5	135.2
	Na	0.4	0.3		0.7		0.7	0.7	1.4
	Ca	24.3	13.6	6.5	44.4	−2.3	42.1	19.9	62.0
	Mg	24.3	11.5	5.6	41.4	−3.1	38.3	27.2	65.5
	Total	77.1	31.1	11.1	119.3	−8.5	110.8	153.3	264.1
−N+N	K	23.0	11.8	6.7	41.5	−2.1	39.4	5.7	45.1
	Na	0.3	0.2		0.5	−0.1	0.4	0.3	0.7
	Ca	18.7	12.2	7.4	38.3	−3.0	35.3	4.1	39.4
	Mg	18.5	13.3	5.9	37.7	−4.0	33.7	6.7	40.4
	Total	60.5	37.5	20.0	118.0	−9.2	108.8	16.8	125.6

S + P + V = Stems + Petioles + Veins

c) Potassium deficiency

Tables 5 and 6 summarize results obtained in a pot experiment on the effect of potassium deficiency on barley. Table 5 shows us that in barley at shooting stage sodium as well as calcium and magnesium replaces a large proportion of potassium.

Analysis at the earing stage shows us that the substitution is more pronounced in the older parts. It should be noted that the balance between the cations is scarcely altered in the ear. Replacement of potassium by calcium and magnesium is also evident in the potato. But, while in barley there is no increase in total cations when K is deficient, this is not the case in potato, a plant which metabolises a high proportion of nitrate in the leaf. However at the first sampling, young potato leaves, which receive their nutrient largely at the expense of the older leaves, can only accumulate small amounts of alkaline earth cations and the sum of cations is thus not augmented in K deficiency.

We think that the increasing in total cations in K deficiency results from the fact that when K is abundant it moves freely and serves in the potato, as in tobacco, for several migration cycles (roots — leaves — roots) that is to say it is concerned more than once in the transport of mineral anions from the roots.

Table 5. (Coïc et al. [5]) Results expressed in milliequivalents per 100 g dry matter (+ K = with added potassium; − K = without added potassium)

	K	Na	Ca	Mg	Total cations
+ K	164	6	56	12	238
− K	77	51	81	22	231
Difference (+ K) − (− K)	87	−45	−25	−10	7

Table 6. (Coïc et al. [5]) Results expressed in meq per 100 g d.m.

		K	Na	Ca	Mg	Total cations
+ K	old parts	79.0	12.6	43.0	10.1	144.7
	young parts	55.7	4.3	32.5	10.8	103.3
	ears	25.7	0.9	8.8	10.0	45.4
− K	old parts	11.8	33.9	68.5	18.3	132.5
	young parts	25.2	11.7	47.0	19.1	103.0
	ears	24.9	1.6	10.9	11.2	48.6

Table 7. Composition of the dry matter of potato leaflets (meq/100 g d.m.)
K_o = plot with potassium deficiency
K = plot rich in potassium

	1st sampling				2nd sampling				3rd sampling			
	Young leaves		Old leaves		Young leaves		Old leaves		Young leaves		Old leaves	
	K_o	K	K_o	K	K_o	K	K_o	K	K_o	K	K_o	K
K	49	79.5	19	82	25	77	15.5	64	15.5	72	6.5	40.5
Ca	61	54.5	295	220	123	64.5	333	299	178	91	342	317
Mg	31.5	26.5	83.5	56	54	29	100	65.5	92	43.5	117	32
Total	141.5	160.5	397.5	358	202	170.5	448.5	428.5	285.5	206.5	465.5	389.5

d) Effect of water shortage

Water deficiency does not affect absorption of all the cations equally: absorption of potassium and magnesium being reduced less than that of calcium and sodium. But the effect of lack of water on cation balance varies with age. Table 8 shows us that when water is deficient the younger parts of barley plants are relatively richer in potassium and markedly lower in calcium.

Table 8. (Coïc et al. [5]) Contents expressed in meq per 100 g d.m.)

		K	Na	Ca	Mg	Total cations
Optimum water supply	old parts	79.0	12.6	43.0	10.1	144.7
	young parts	55.7	4.3	32.5	10.8	103.3
	ears	25.7	0.9	8.8	10.0	45.4
Water shortage	old parts	74.2	6.2	47.5	13.1	141
	young parts	63.6	1.7	20.0	12.4	97.7
	ears	30.8	0.7	8.5	11.6	51.6

e) Influence of root temperature

Refrigeration of maize roots to 10–12° compared with a normal temperature of 20–25° inhibits reduction of nitrate in the root; but the depression of respiratory metabolism causes other modifications with complex consequences. Nevertheless so far as we are concerned one finds that the leaf metabolises a greater proportion of absorbed nitrate (increase in organic acid content of the dry matter) and the contents and proportions of calcium found in young or adolescent leaves increase while K content diminishes.

Table 9. (Coïc et al. [9])

		Young leaves		Adolescent leaves	
		Normal temperature	Low temperature	Normal temperature	Low temperature
Organic acid concentration (meq/100g fresh weight)		5.4	8.2	7.6	14.1
Proportion of each cation (% of total)	K	78.5	76.5	55	49.2
	Na	0.3	0.6	0.2	0.4
	Ca	6.8	8.7	19.8	26.9
	Mg	14.4	14.2	25	23.5
		100	100	100	100

f) Effect of cationic composition of the medium on cationic composition of the leaves (Coïc et al. [8])

The effect of change in the cationic composition of the growing medium varies greatly according to plant species. In plants like maize, whose roots metabolise nitrate to a great extent, there is a kind of selectivity in the transport of cations to the leaves while in tomato the competition between different cations has a much more evident effect upon leaf composition. Thus high Ca concentration (8 meq/1; Mg = 1, K = 1) in comparison to equal concentration of all three (1 meq/1) and high Na has very different effects on leaves of tomato and maize: the contents of Mg of the maize leaves remain approximately the same, while those of the tomato leaves are strongly reduced by the high Ca concentration in the nutrition medium.

Table 10. (Coïc et al. [8]) Mineral content of leaf blades (meq per 100 g d.m.)

		Young leaf blades		Old leaf blades	
		high Na	high Ca	high Na	high Ca
Maize	K	148	144.5	141	123.5
	Na	1.7	0.8	2.6	0.7
	Ca	6.1	14.6	26.9	48.9
	Mg	20.8	19	27.8	26.4

		Young leaflets		Adult leaflets		Old leaflets	
		high Na	high Ca	high Na	high Ca	high Na	high Ca
Tomato	K	103	102	111	96	112	92.5
	Na	11	1	21	1.2	31	1.5
	Ca	60.5	137	118.5	241	175	299
	Mg	50	30	78	41	111	59

3. *Cationic composition of other organs. (Coïc et al. [6])*

The cationic composition of organs depends essentially upon the role which they play in the physiology of the plant. Organs containing chlorophyll and particularly the leaf are concerned with photosynthesis. The roots and leaves are implicated in mineral metabolism, in particular of NO_3^- and NH_4^+. Stems, petioles and veins are concerned with the transport of mineral and organic substances. Fruits, grain, tubers, bulbs, certain roots and rhizomes are storage organs accumulating organic material. This is how we regard in a general way the processes of accumulation. The accumulation of mineral cations in an organ represents the balance between ions coming into and leaving that organ.

1) Incomings:
Mineral cations reach the organ together with mineral anions and/or organic substances. Among the mineral anions, NO_3^- is the most important on a quantitative basis. Organic substances concerned in transport are the proteids, mainly amino-acids, glucides (hexoses, holosides and sugar phosphates) and in certain circumstances to a lesser degree organic acids. K is the cation mainly associated with the transport of organic substances and magnesium is concerned to a lesser degree.

Table 11. (Coïc et al.[6]) Cation content of various organs of young tomato plants (meq/100 g fresh weight)

	K	Ca	Mg
Leaflets	6.8	16.0	8.8
Stems and Petioles	8.5	2.7	2.6
Roots	4.7	0.7	1.5

Table 12. (Coïc et al.[6]) Mineral composition of tomatoes (comparison of fruits and leaves) meq/100 g d.m.)

	Young leaves	Old leaves	Small fruit (green)	Large fruit (green)
K	54.9	71.2	103.8	104.5
Ca	89.8	341.0	9.2	7.1
Mg	32.0	76.3	20.4	35.2

Table 13 (Coïc et al. [6]) Mineral composition of carrots (comparison of leaves and storage roots) meq/100 g d.m.

	Leaves	Roots
K	170	116
Ca	93.5	16.5
Mg	32.8	20.2

Table 14. (Coïc et al.[6]) Mineral composition of potatoes [comparison of leaves (young and old) and tubers] meq/100 g d.m.

	Young leaves	Old leaves	Tubers
K	60.5	46	45
Ca	152	258	2
Mg	60.5	53	7.6

2) Outgoings:

We have spoken about the accumulation of cations, as salts or organic acids, in the vacuoles of leaf cells as a consequence of the metabolism of nitrate. But there are other ways in which they are retained in the cell. Of a physico-chemical nature by absorption on the polyuronic acids of the cell wall, on the chloroplasts or cytoplasmic colloids; and of a chemical nature by the formation of compounds in reserve organs (Mg and Ca phytate for example).

We can say in general that organs which feed mainly upon organic substances will be particularly rich in potassium and poor in calcium. This is true for the young organs during growth, stems, fruits, tubers, storage roots, etc. We shall give several examples *(Coïc et al. [6]).*

Tables 15 and 16 are interesting in that they demonstrate that the tumors on the stems or leaves caused by ***Bacterium tumefaciens*** are sharply distinguished in their mineral composition from the organs which carry them since they receive an organic food supply carrying much potassium.

It is well known *(Isidore-Pierre, [14], Coïc et al. [3])* that the nodes of cereal plants are particularly rich in mineral cations. Table 17 shows us once more that potassium deficiency affects the nodes less than other parts of the plant.

Special case of the accumulation of sodium

The problem of the accumulation of sodium in plant organs does not seem to have been resolved, particularly the capability of some plants (beet, endive, barley ...) to accumulate Na in their aerial parts while one does not find such accumulation in other plants. But in

Table 15. (Coïc et al. [6]) Comparative mineral composition of leaves and leaf borne tumors of *Bryophyllum* (meq per 100 g d.m.)

	K	Na	Ca	Mg
Leaves	76.5	3.5	391	49.7
Tumors	55	1	72.6	17.7

Table 16. (Coïc et al. [6]) Comparative mineral composition of stems and stem borne tumors of *Bryophyllum* (meq per 100 g d.m.)

	K	Ca	Mg
Stems	89.5	99.5	38.2
Tumors	98	50	31.6

Table 17. Cation contents of different parts of barley plants grown on potash deficient (K_o) and non-deficient (K) soil (meq per 100g d.m.)

	Nodes		Inter-nodes		Sheathes		Leaf blades	
	K_o	K	K_o	K	K_o	K	K_o	K
K	73.9	155	27.9	82.1	31.2	96.2	28.7	89.1
Na	9.8	4.3	8.5	5.9	6.1	3.5	3.7	3.1
Ca	13.9	12.4	6	7.1	30.1	26.6	91	55
Mg	33.7	19.7	13.9	9.9	26.3	18.1	28.7	23
Total	131.3	191.4	56.3	105	93.7	144.4	152.1	170.2

the latter kinds one can find considerable accumulations in the roots. There are two possible hypotheses *(Barbier* and *Chabannes [2])* either penetration of Na across the root tissue towards the vessels and its transport upwards is impeded more than that of potassium with the result that Na accumulates in the lower portions; or, on the contrary, Na is more mobile, passes through all parts of the plant more easily and go down again to be accumulated in the roots. To decide this the authors grew peas in solution cultures divided into two compartments so that sodium was supplied to only one half of the root system (0.6 meq/l). Much Na accumulated in the roots not directly supplied with Na.
Of the two hypotheses suggested it appears that the second, 'accumulation per descensum,' should be adopted.

Table 18. (Barbier et Chabannes [2])

	Roots with Na	Stems and leaves	Roots without Na
1) 0.1 meq K per litre of solution			
meq Na per 100 g	30	4.6	26.7
Ratio Na/K	1.5	0.12	0.87
2) 0.5 meq K per litre of solution			
meq Na per 100 g	21.5	4.8	16.4

Conclusion

The physiological processes which govern the accumulation of a mineral cation in plant organs are still relatively poorly understood. We have emphasised those concerned with NO_3-metabolism and its localisation. The processes of transport of cations with organic substances play a fundamental role. The behaviour of sodium is controversial.

Bibliography

1. *Arnon, D.I.:* Effect of ammonium and nitrate nitrogen on the mineral composition and sap characteristics of barley. Soil Sci. *48*, 295–307 (1939).
2. *Barbier, G.*, and *Chabannes, J.:* Accumulation du sodium dans les racines des plantes. C.R. Acad. Sci. *232*, 1372–1374 (1951).
3. *Coïc, Y., Lesaint, C.*, and *Le Roux, F.:* Comparaison des feuilles, entre-nœuds et nœuds du Blé au point de vue acides organiques et cations minéraux. Ann. Physiol. vég. *3*, 1, 33–37 (1961).
4. *Coïc, Y., Lesaint, C.*, and *Le Roux, F.:* Comparaison de l'influence de la nutrition nitrique et ammoniacale, combinée ou non avec une déficience en acide phosphorique, sur l'absorption et le métabolisme des anions-cations et plus particulièrement des acides organiques chez le maïs. Comparaison du maïs et de la tomate quant à l'effet de la nature de l'alimentation azotée. Ann. Physiol. vég. *3*, 3, 141–163 (1961).
5. *Coïc, Y., Lesaint, C.*, and *Piollat, M.T.:* Influence de la déficience en potassium et de la déficience en eau sur la composition minérale de l'orge. Ann. Physiol. vég. *4*, 3, 227–234 (1962).
6. *Coïc, Y., Lesaint, C.*, and *Grandjean, M.:* Sur la composition minérale des espèces et organes végétaux et leur déterminisme. Ann. Physiol. vég. *5*, 4, 293–301 (1963).
7. *Coïc, Y.:* Sur le déterminisme de l'absorption des cations minéraux par les genres et espèces végétales: influence de la localisation du métabolisme de l'azote. C.R. Acad. Agric. Fr. *50*, 925–932 (1964).
8. *Coïc, Y., Lesaint, C., Provot, M.*, and *Le Roux, F.:* Comparaison de l'antagonisme des cations dans la nutrition minérale du maïs et de la tomate. Ann. Physiol. vég. *8*, 2, 87–100 (1966).
9. *Coïc, Y., Lesaint, C.*, and *Le Roux, F.:* Influence du refroidissement des racines sur l'absorption, le métabolisme des ions et l'équilibre anions-cations chez le maïs. Ann. Physiol. vég. *8*, 4, 313–320 (1966).

10. *Coïc, Y., Lesaint, C.,* and *Grandjean, M.:* Sur le déterminisme de l'alcalinisation physiologique et sa variation suivant les espèces végétales. C.R. Acad. Agric. Fr. *55*, 4, 291–297 (1969).
11. *Coïc, Y., Lesaint, C., Piollat, M.T.,* and *Lelandais, M.:* Effet de la nutrition azotée nitrique sur l'évolution quantitative et qualitative des organes aériens du Tabac. Ann. Physiol. vég. *11*, 1, 5–26 (1969).
12. *Coïc, Y., Lesaint, C., Piollat, M.T.,* and *Lelandais, M.:* Evolution de l'accumulation des acides organiques dans les limbes de feuilles de Tabac d'âges différents comme conséquence du métabolisme des nitrates *in situ* C.R. Acad. Sci. *270*, D, 2280–2283 (1970).
13. *Coïc, Y., Lesaint, C., Piollat, M.T.,* and *Lelandais, M.:* Effet de la nutrition azotée nitrique sur l'évolution des cations K, Ca, Mg, dans les organes aériens du Tabac. Rev. Potasse, *sect. 3*, 38e suite, 8 p. (1970).
14. *Isidore-Pierre, J.:* Etudes expérimentales et analytiques sur les migrations de la potasse dans le froment. Ann. Agron. *2*, 59–72 (1876).

Inorganic Cations and Carboxylates in Young Sugar-Beet Plants

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Summary

It is shown in a field experiment, that in sugar-beet *(Beta vulgaris* L.) plants, supplied with nitrate as source of nitrogen, the amount of meq. (inorganic cations – inorganic anions) is equal to that of carboxylates (organic anions) and to the amount of m mole organic N. The distribution of organic N and of carboxylates over the plant leads to the conclusion that the organic N was transported from elder to younger leaves. The cations Na^+, Ca^{2+} and Mg^{2+} accumulate in the aerial sections of the sugar-beet plant in equivalent quantities with oxalate-ions. The Na-K substitution-effect in sugar-beet is explained on the strength of experimental material presented here.

1. *The aim of research*

It is generally assumed that the difference between the total sum of cations and inorganic anions in plant material is rather specific for different plant species. This difference is compensated by the presence of carboxylates.
The aim of this research is to study the content and transport of ions between the different parts of the sugar-beet plant *(Beta vulgaris* L.) as it takes place during the vegetative stage under field conditions. Special attention is paid to the part which can be played in this transport by the carboxylates ($RCOO^-$).
Nitrogen dressings are included in this study because of the effect of nitrate-utilization on the formation of carboxylates.

2. *Experimental*

2.1 *Field experiment*

On the 2nd of May 1969 an experimental site was prepared on a light coloured, gravelly sandy soil, poor in organic matter*. Following doses of fertilizers (kg/ha) had previously been applied to this field:

133 kg K	(160 kg K_2O)	as 400 kg of muriate of potash 40%
83 kg P	(84 kg P_2O_5)	in the form of 600 kg of basic slag
and	(108 kg P_2O_5)	in the form of 600 kg of superphosphate
45 kg Mg	(50 kg MgO)	as 200 kg of kieserite
and	(25 kg MgO)	in the dolomitic-limestone
174 kg Ca	(435 kg $CaCO_3$)	as 500 kg dolomitic-limestone

* Wageningen-Hoog experimental fields of the Department of Field Crops and Grassland Husbandry of the University.

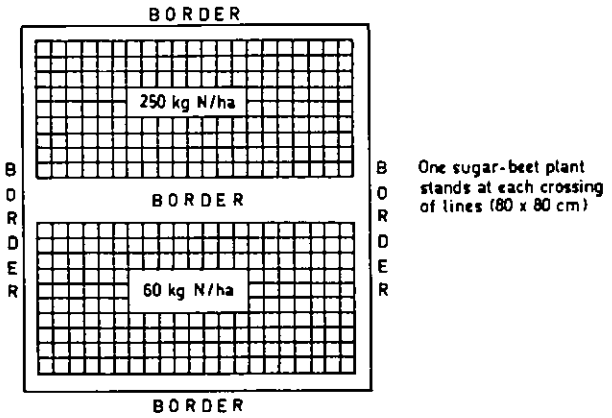

Figure 1. Scheme of experimental field.

When the site was ready for sowing 60 kg of N/ha were applied in the form of nitrate of lime. Per hectare 20 kg of borax were applied to prevent the occurence of B-deficiency. Pyramin was applied as pre-emergence weed killing spray.
Diploid sugar-beet seed was drilled at plant distance 80 × 80 cm, 3 seeds per hole. After emergence of the crop one half of the field was top dressed with 190 kg of N per ha in the form of nitrate of lime. In figure 1 the schema of experimental field is shown that was obtained after above mentioned treatments.

2.2 *Maintenance of the crop and harvesting procedure*

After the emergence the number of plants was reduced to one plant per hole. Owing to the wide spacing the field had to be hoed frequently to suppress the weeds. The open stand also promoted infection by lice and the crop was therefore sprayed at regular intervals.
The soil being rather susceptible to drought several spray irrigations were applied in the course of the sunny and dry season of 1969.
This paper is based on analysis of samples harvested on the 3rd of July 1969. Each sample consisted of ten plants collected at random from each treatment. After harvesting, the plants were divided into the following sections: leaf blades, petioles, tops and roots. During the growth of the crop the number of leaves was regularly counted and leaves of the same numerical order marked with plastic strings. Figure 2 illustrates the way of sampling. Blades of leaves of the same numerical order from ten harvested sugar-beet plants were mixed together to form one sample. Similarly the corresponding petioles formed a sample bearing the same number of the leaf. Sample A consists of ten root tops, sample B of ten roots.
After determining the fresh weight, all the samples were dried at 70 °C for 24 hours, weighed again and finely ground for chemical analysis.

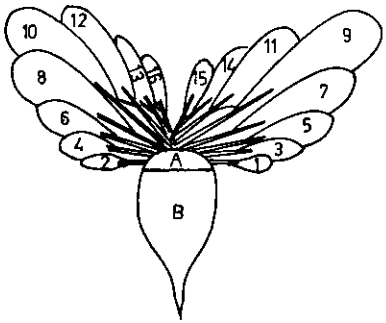

Figure 2. The location of the separate samples in the sugar-beet plant.

2.3 *Laboratory methods*

Samples were analysed for Na, K, Ca, Mg, H_2PO_4, NO_3, Cl, SO_4, total N, organic acids (i.e. oxalic, malic acid, citric acid, fumaric acid, succinic acid and malonic acid). Subsamples were analysed after digestion in concentrated sulphuric acid and hydrogen peroxide in the presence of salicylic acid. In this digest, the Na, K and Ca contents were determined flamephotometrically, the H_2PO_4 content colorimetrically, the Mg content by atomic absorption and the nitrogen content (total N) by the micro-Kjehldahl procedure. Other subsamples were extracted with water (0,5 g of plant material and 50 ml deionized water: shaking time 2 hours). In this extract the nitrate-content was determined with an Orion nitrate electrode, the Cl content was determined coulometrically with a chloro-counter and the SO_4 content colorimetrically. The total content of carboxylates (C-A) was calculated by substracting the sum of inorganic anions from the sum of inorganic cations *(de Wit et al. [1])*. The carboxylates were converted into organic acids by decationization with a H^+ sulphonic acid resin, these then were resolved by partition chromatography and quantitatively determined with an automatic titrator.
Since the treatment did not extract all the oxalates, the remaining oxalates were determined oxidimetrically with $KMnO_4$ following a double treatment of the samples with 2 *N* HCl. (In the blank determination hardly any $KMnO_4$ was used.)

3. *Results and discussion*

3.1 *(C-A) value as compared with the sum of carboxylates*

It is generally assumed, that the difference between the total sum of inorganic cations and the sum of inorganic anions found in plant material is compensated by the presence of carboxylates [*2, 3, 4, 5, 6, 7, 8*]. Therefore there should be at least two methods to determine the total sum of carboxylates, e.g.

a) by separate determinations of the inorganic cations and anions and substracting the latter from the former according to the formula C-A =(meq K^+ + meq Na^+ + meq Ca^{2+} + meq Mg^{2+})-(meq Cl^- + meq NO_3^- + meq $H_2PO_4^-$ + meq SO_4^{2-}).

In case other inorganic ions should be present in considerable quantities they also must be included in the above calculation.

b) by determining seperately the different carboxylates and summing up the found values.

Determination of the difference C-A has the advantage of being done quickly and very accurately. The disadvantage is that only the total amount of carboxylates is found in this way without any information on its component parts.

Seperate determination of carboxylates has the advantage of providing information on quality and quantity of different carboxylates in a given sample. For the study of this subject this fact is of great importance because of differences between the nature of the various $RCOO^-$-groups. The disadvantage of this method is its laboriousness and therefore its high cost. In view of the object of this study it was quite natural to use both methods because the inorganic cations and the carboxylates had to be determined anyhow. By using both methods it was possible to compare the direct determination of carboxylates with the calculation of (C-A) as described above.

The material available in the form of leaf blades and petioles shows a great variability in the carboxylate contents and in the (C-A) value as well, which makes it very suitable for calculation of the regression function between (C-A) and the anions of water-soluble organic acids. Results of this calculation are given in Table 1; in Figure 3 all the data are represented in a graph.

Table 1. The relation between (C–A) and carboxylates

Fertilizer dose	Number of samples	Regression equation, $y = a + bx$	Correlation Coefficient
250 kg N/ha	16 leaf blades	C–A = −740 + 1.17 [Σ (carboxylates)]	r = 0.99
250 kg N/ha	16 petioles	C–A = −410 + 1.04 [Σ (carboxylates)]	r = 0.98
60 kg N/ha	15 leaf blades	C–A = 110 + 1.05 [Σ (carboxylates)]	r = 0.97
60 kg N/ha	14 petioles	C–A = −170 + 1.00 [Σ (carboxylates)]	r = 0.92
Total	61	C–A = −410 + 1.11 [Σ (carboxylates)]	r = 0.97

(C–A) and carboxylates in meq per kg dry matter

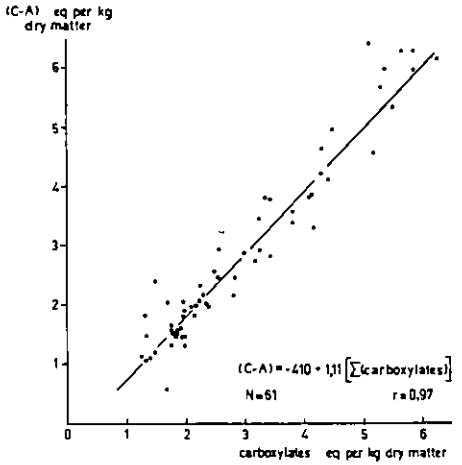

Figure 3. Relation between carboxylates and (C-A).

The high correlation coefficients leave no doubt about a correlation existing between the value of (C-A) and the total amount of carboxylates. It is also clear that the value of (C-A) increases by practically one unit when the total amount of carboxylates show a one unit increase.

Freeman [9] pointed out, that high NO_3^--contents in samples may cause too high values to be found when determining malate and citrate. It is noticeable that in the 'high-N' group the sum of carboxylates is always somewhat higher than the value for (C-A). Since very high NO_3^--contents are found, in this material, this phenomenon seems to be in agreement with the disturbance as described by *Freeman*.

In general terms it can be stated that the following equation holds for both the leaf blades and petioles of the sugar-beet plant at this stage of development:

$(C\text{-}A) = \Sigma$ (carboxylates)

wherein both terms are expressed in equivalents.

3.2 *Relation between the total amount of (C-A) and of organic nitrogen in the plants*

Several scientists have studied the factors which may affect the value of (C-A) as found in crop samples [2, 10, 11, 7]. *Houba et al.* [12] give a review of the processes influencing the carboxylate pool (Table 2).

When plants are supplied with nitrate-N it is the process of NO_3^--reduction which mainly determines the amount of formed carboxylates. Other processes given in Table 2 can cause appreciable difference between the amount of meq of the total of carboxylates and of mmole of organic N. For sugar-beet grown in nutrient solution, *Houba et al.* [12] have shown that for plants grown on nitrate as the only source of nitrogen, the reduction of NO_3^- determines both the amount of organic N and the total sum of carboxylates as well. It appears therefore, that under these conditions only the process No. 3 plays a part of importance. The summing-up of the figures which give the contributions from different parts of the plants for the harvest discussed here, leads to the same conclusion, as is demonstrated in Table 3. In Figure 4 data from *Houba et al.* [12], *van Egmond et al.* [13] and this experiment are represented together.

Table 2. Processes which influence the carboxylate pool

Process	Carboxylate
1. Uptake of $Na^+ + K^+ + Ca^{2+} + Mg^{2+}$ meq < uptake of $NO_3^- + Cl^- + SO_4^{2-} + H_2PO_4^-$ meq	decrease
2. Uptake of $Na^+ + K^+ + Ca^{2+} + Mg^{2+}$ meq > uptake of $NO_3^- + Cl^- + SO_4^{2-} + H_2PO_4^-$ meq	increase
3. Nitrate reduction	increase
4. Sulphate reduction	increase
5. Transition of NH_4^+ into organic N	decrease

Table 3. Total amount of organic N and (C-A)

Fertilizer dose	250 kg N/ha		60 kg N/ha	
Section	Organic N in mmole	C-A in meq	Organic N in mmole	C-A in meq
Leaf blade	235	257	186	188
Petiole	30	40	23	37
Top	12	2	4	2
Root	30	17	24	9
Total	307 mmole/10 pl	316 meq/10 pl	237 mmole/10 pl	236 meq/10 pl

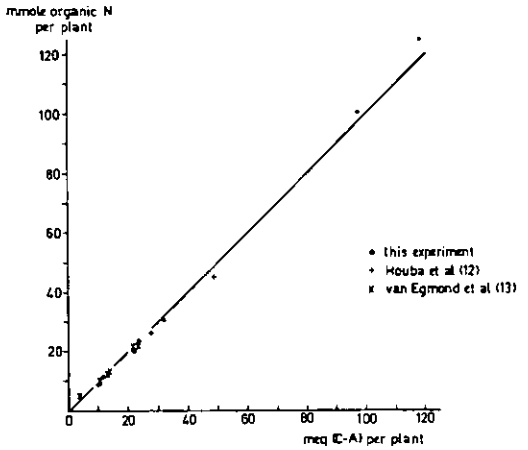

Figure 4. Relation between organic N and (C-A) in sugar-beet plants.

The conclusion must be that in young and normally dressed sugar-beet plants the amount of organic N corresponds with the amount of carboxylates. This again indicates that under given conditions young sugar-beet plants show a neutral ion absorption, which observation is in accordance with the findings described in [*12*].

3.3 *Age of leaves, (C-A) and organic-N content*

Taking the plant as a whole, it is shown that the value of C-A ≅ organic N. However this agreement does not hold when separate parts of the plant are considered. In figures 5, 6, 7 and 8 values of (C-A), sum of carboxylates and organic N are plotted against the leaf number (age). They show clearly the content of organic N decreases with age while the sum of carboxylates, on the contrary, shows a strong increase. Inorganic cations reach the leaf mainly together with NO_3 and are then partly tied up by non-mobile $RCOO^-$ groups. These carboxylates accumulate in the leaf. Organic N appears to be very mobile and is transported from elder into younger leaves. This is shown by the fact that in old leaves C-A > organic N while the reverse is the case in young leaves. In this line of reasoning (C-A) is considered as a slag formed during the process of the reduction of nitrate.

3.4 *Distribution of the cations and anions over the various organs of the sugar-beet plant*

The fact that values found for (C-A) depend on leaf age and the knowledge that the contents of cations and anions in plants also may vary from place to place give reason to compile the contents of cations and anions in absolute and relative sense as they were found in different sections of the plants. These data are shown in Table 4 and Figure 9 and 10.

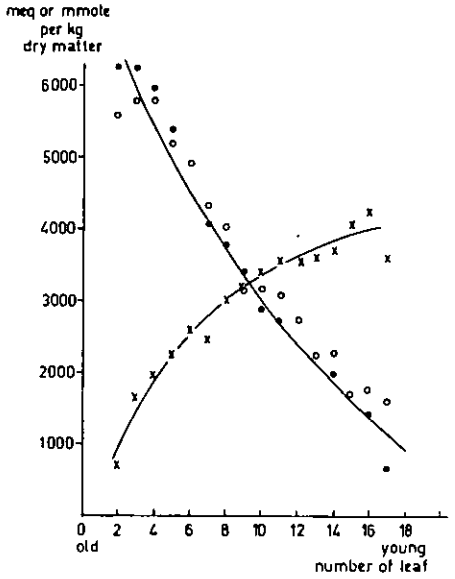

Figure 5. Relation between number of leaf and organic N (x), C-A (.), sum of carboxylates (o). Fertilizer dose 250 kg N/ha.

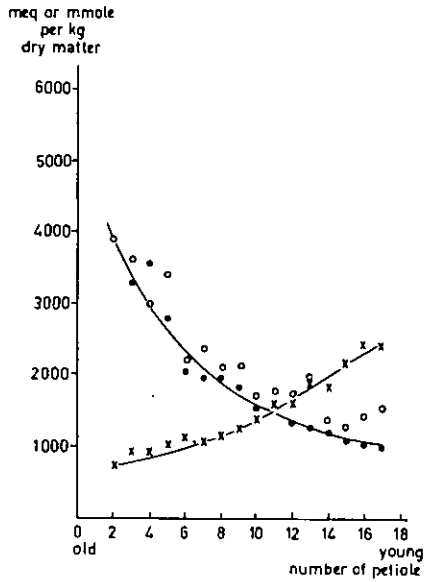

Figure 6. Relation between number of petiole and organic N (x), C-A (.), sum of carboxylates (o). Fertilizer dose 250 kg N/ha.

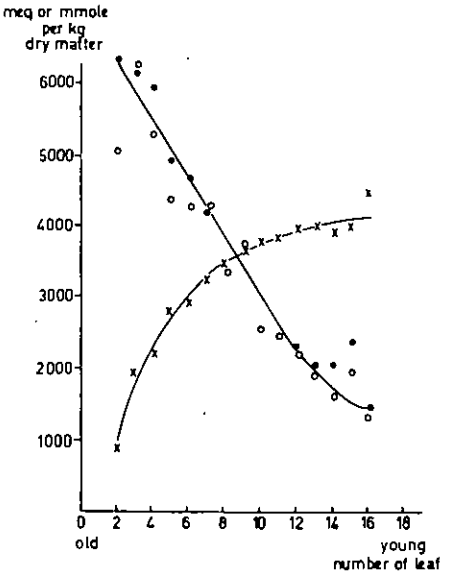

Figure 7. Relation between number of leaf and organic N (x), C-A (.), sum of carboxylates (o). Fertilizer dose 60 kg N/ha.

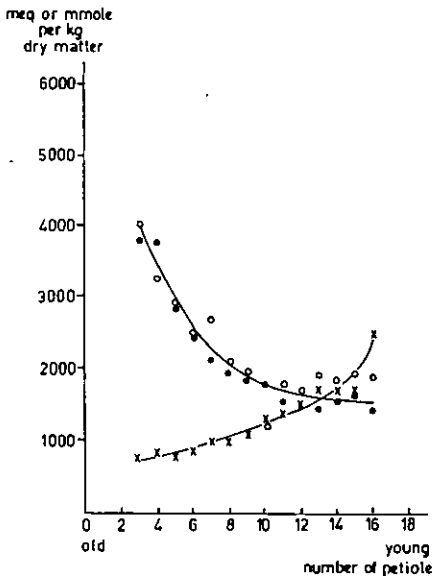

Figure 8. Relation between number of petiole and organic N (x), C-A (.), sum of carboxylates (o). Fertilizer dose 60 kg N/ha.

Table 4. Distribution of the ions over the various organs of the sugar-beet plant. Amounts in meq per plant. Plants 45 days old

Section	Na^+		K^+		Ca^{2+}		Mg^{2+}		Cl^-		NO_3^-		SO_4^{2-}		$H_2PO_4^-$		C–A		*		Oxalate		**	
	meq	%	meq	%	meq	%	meq	%	meq	%	meq	%	meq	%	meq	%	meq	%	meq	%	meq	%	meq	%
Fertilizer dose: 250 kg N/ha																								
a.	2.74	76.0	9.37	58.0	9.40	83.4	8.41	83.8	0.25	38.2	2.84	41.0	0.15	42.8	1.02	66.5	25.67	81.4	27.00	79.6	23.17	83.6	2.83	54.6
b.	0.63	17.4	4.35	27.1	1.60	14.2	1.10	11.0	0.32	49.8	3.00	43.3	0.10	29.5	0.27	17.8	3.98	12.6	4.66	13.8	3.24	11.7	1.42	27.4
c.	0.04	1.2	0.32	1.9	0.04	0.3	0.09	0.9	0.02	2.7	0.18	2.5	0.03	8.6	0.04	2.8	0.23	0.7	0.44	1.3	0.32	1.2	0.11	2.2
d.	0.20	5.5	2.04	12.7	0.23	2.0	0.43	4.3	0.06	9.4	0.91	13.2	0.06	19.2	0.20	13.0	1.66	5.3	1.81	5.3	0.99	3.6	0.82	15.8
Total	3.61		16.09		11.27		10.03		0.65		6.93		0.34		1.53		31.55		33.91		27.73		5.18	
Fertilizer dose: 60 kg N/ha																								
a.	2.60	75.1	7.21	59.2	6.26	80.7	5.17	82.7	0.16	29.2	1.09	29.4	0.35	67.4	0.84	64.3	18.79	79.8	17.80	76.1	15.35	81.0	2.45	55.2
b.	0.64	18.6	3.80	31.2	1.25	16.1	0.72	11.5	0.34	60.1	2.11	57.0	0.09	16.9	0.22	16.6	3.66	15.6	3.96	16.9	2.55	13.4	1.41	31.8
c.	0.03	0.8	0.16	1.4	0.06	0.7	0.05	0.9	0.01	2.1	0.06	1.7	0.02	2.9	0.03	2.3	0.18	0.8	0.33	1.4	0.26	1.4	0.07	1.6
d.	0.19	5.6	1.00	8.2	0.19	2.5	0.31	5.0	0.05	8.6	0.44	11.9	0.07	12.8	0.22	16.8	0.92	3.9	1.30	5.6	0.80	4.2	0.51	11.4
Total	3.46		12.17		7.76		6.25		0.56		3.70		0.53		1.31		23.55		23.39		18.95		4.44	

Section: a. = leaf blade b. = petiole c. = top d. = root * = Σ(Carboxylates) ** = Σ(Carb.)-Oxalate

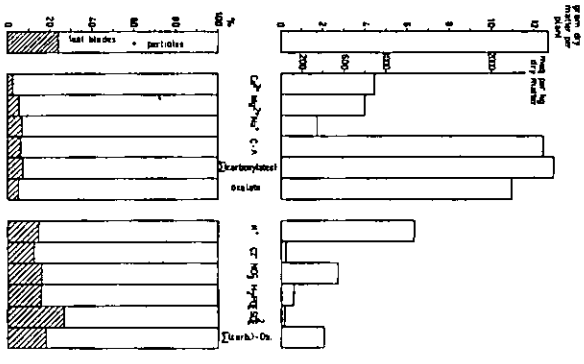

Figure 9. Average content and distribution of the ions over aerial and subterranean part of 45 days old sugarbeet plants. Fertilizer dose 250 kg N/ha.

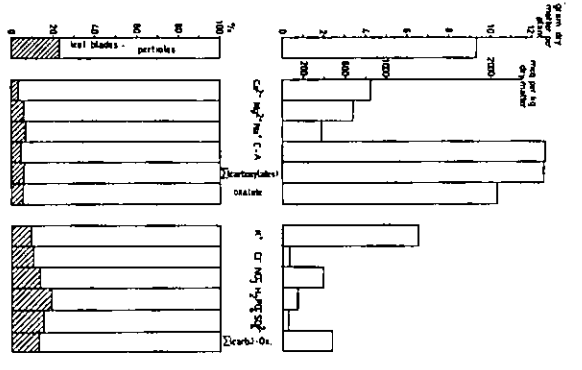

Figure 10. Average content and distribution of the ions over aerial and subterranean part of 45 days old sugarbeet plants. Fertilizer dose 60 kg N/ha.

Comparing the total contents of cations K^+, Ca^{2+} and Mg^{2+} in the plant with those of the anions Cl^-, SO_4^{2-} and $H_2PO_4^-$ it appears that in all the sections the former are present in much greater quantities than the latter. Correspondingly the figures for (C-A) show a great excess of inorganic cations which is compensated by the sum of carboxylates. Oxalate is the most prevailing organic anion.

The distribution of the components is very much affected by the pattern in which the dry matter, as formed by the plant, is distributed over its different sections at this stage of development. Table 5 shows this distribution of the dry matter.

Considering the sections of leaf blades and petioles as aerial part and the sections tops and roots as subterranean parts of the plants, it appears that irrespective of the N-dose the subterranean parts constitute 23—24 per cent of the total amount of dry matter produced by these young plants. Almost all the ions show a distribution whereby the greatest share of the total quantity is located in the aerial parts. The best similarity between the distribution of the ion and dry matter is found with the ions K^+, Cl^-, NO_3^-, SO_4^{2-}, $H_2PO_4^-$ and (Σ [carboxylates] − oxalate). 10—30 per cent of the total amount of these components is located in the subterranean sections. Na^+, Ca^{2+}, Mg^{2+}, Σ(carboxylates), and oxalate show a markedly different distribution: less then 10 per cent is contained in the subterranean sections, in some instances like Ca^{2+} even not more than 2—3 per cent. Accumulation of the ions Na^+, Ca^{2+}, Mg^{2+} and oxalate obviously shows figures of the same magnitude.

Table 5. Distribution of dry matter over the different sections of 45 days old sugar-beet plants.

Section	Nitrogen dressing			
	250 kg N/ha		60 kg N/ha	
	gram	%	gram	%
Leaf blade	7.49	59.0	5.32	57.0
Petiole	2.18	17.4	1.83	19.7
Top	0.53	4.2	0.23	2.5
Root	2.50	19.4	1.92	20.8
Total	12.7		9.3	

3.5 *Relation between inorganic cations and the various anions*

Accumulation of Ca^{2+}, Mg^{2+} and Na^+ in the aerial parts of plants is a phenomenon which has often been observed. Data described in the previous chapters justify the presumption that certain oxalates are accumulated in the foliar tissues. It should be mentioned however, that a connection found between a cation and oxalate does not necessarily mean, that the accumulation of the cation is caused by oxalate. Most obvious is the correlation between the sum of cations (C) and the oxalate because in this material the former practically corresponds with the absorbed amount of NO_3^- while the amount of oxalate almost equals the amount of reduced NO_3^-.

When the foliage is constantly supplied with cations in one and the same ratio and the cations are not carried off, a connection is to be expected between the quantity of inorganic cations and oxalate.

In Figure 11 the content of the different cations is plotted against that of oxalate, while Table 6 shows the regression equations and correlation coefficients for the different cations and oxalate.

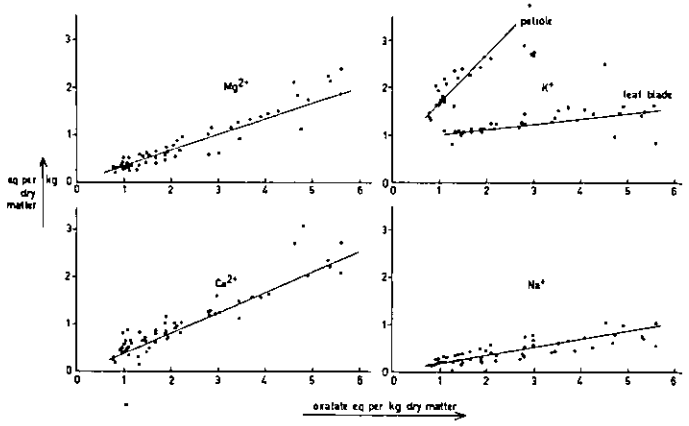

Figure 11. Relation between K^+, Na^+, Ca^{2+}, Mg^{2+} and oxalate in leaf blades and petioles of 45 days old sugarbeet plants.

Table 6. Compilation of regression functions between inorganic cations and oxalate ($y = a + bx$)

Fertilizer dose	250 kg N/ha		60 kg N/ha		
Number of samples	meq per kg d.m.	Correlation coefficient	Number of samples	meq per kg d.m.	Correlation coefficient
16 Lb	$Na^+ = -70 + 0.14$ (Ox)	0.96	15 Lb	$Na^+ = -70 + 0.20$ (Ox)	0.96
15 p	$Na^+ = -20 + 0.23$ (Ox)	0.98	14 p	$Na^+ = -70 + 0.30$ (Ox)	0.98
16 Lb	$K^+ = 980 + 0.07$ (Ox)	0.52	15 Lb	$K^+ = 1010 + 0.10$ (Ox)	0.61
15 p	$K^+ = 1440 + 0.36$ (Ox)	0.75	14 p	$K^+ = 1240 + 0.57$ (Ox)	0.91
16 Lb	$Ca^{2+} = -340 + 0.51$ (Ox)	0.99	15 Lb	$Ca^{2+} = 0 + 0.42$ (Ox)	0.93
15 p	$Ca^{2+} = -150 + 0.66$ (Ox)	0.97	14 p	$Ca^{2+} = 10 + 0.45$ (Ox)	0.90
16 Lb	$Mg^{2+} = -270 + 0.45$ (Ox)	0.99	15 Lb	$Mg^{2+} = -60 + 0.37$ (Ox)	0.95
15 p	$Mg^{2+} = 220 + 0.20$ (Ox)	0.96	14 p	$Mg^{2+} = 180 + 0.15$ (Ox)	0.93
16 Lb	$Na^+ + Ca^{2+} + Mg^{2+} = -700 + 1.11$ (Ox)	0.99	15 Lb	$Na^+ + Ca^{2+} + Mg^{2+} = 90 + 0.99$ (Ox)	0.96
15 p	$Na^+ + Ca^{2+} + Mg^{2+} = 50 + 1.10$ (Ox)	0.98	14 p	$Na^+ + Ca^{2+} + Mg^{2+} = 120 + 0.89$ (Ox)	0.94

Lb = leaf blade p = petiole

Table 7. Regression functions between K^+ and the sum of anions-oxalate ($y = a + bx$)

Number of samples	meq per kg d.m.	Correlation coefficient
Fertilizer dose: 250 kg N/ha		
16 Lb	$K^+ = 620 + 0.59\,[NO_3^- + Cl^- + SO_4^{2-} + H_2PO_4^- + \Sigma\,(Carb.\text{-}Ox)]$	r = 0.66
16 p	$K^+ = 250 + 0.71\,[NO_3^- + Cl^- + SO_4^{2-} + H_2PO_4^- + \Sigma\,(Carb.\text{-}Ox)]$	r = 0.93
Fertilizer dose: 60 kg N/ha		
15 Lb	$K^+ = 420 + 1.02\,[NO_3^- + Cl^- + SO_4^{2-} + H_2PO_4^- + \Sigma\,(Carb.\text{-}Ox)]$	r = 0.71
14 p	$K^+ = 620 + 0.67\,[NO_3^- + Cl^- + SO_4^{2-} + H_2PO_4^- + \Sigma\,(Carb.\text{-}Ox)]$	r = 0.70

Lb = leafblade p = petiole

It appears that irrespective of the nitrogen dose, the contents of Na^+, Ca^{2+} and Mg^{2+} in both leaf blades and petioles are closely correlated with the oxalate content, the same is the case when the sum of these cations is considered whereby it is to be noted that the increase of the sum of $Na^+ + Ca^{2+} + Mg^{2+}$ is equivalent to that of the oxalate (see Figure 12). This suggests a combined accumulation.

On the contrary, the potassium content does not show a high degree of correlation with the oxalate content (with the exeption of petioles where a high correlation coefficient is calculated at the dose of 60 kg of N per ha). Obviously the K-content depends only slightly on the oxalate content. Similar calculations have been made too on Spinach *(Spinacia oleracea* L.) by *Bengston et al.* [8]. Our experiments suggest that the correlation coefficient as calculated by these authors between the $K^+ + Na^+ + Ca^{2+} + Mg^{2+}$ content and the oxalate content will be lower than the correlation coefficient between $Na^+ + Ca^{2+} + Mg^{2+}$ content and the oxalate content.

When NO_3^- supply is sufficient Na^+, Ca^{2+} and Mg^{2+} appear to accumulate in the foliage of the sugar-beet, together with the oxalate, in a 1 to 1 ratio. Occasionally K^+ can be accumulated together with the oxalate as, for instance was observed in the low calcium nitrate treatment.

Equivalency between the sum of $Na^+ + Ca^{2+} + Mg^{2+}$ and the amount of oxalate suggests that the potassium ion content should be correlated with the sum of inorganic iones and Σ(carboxylates – oxalate).

Table 7 indicates that potassium which is evenly distributed in the plant, shows a correlation with the amount of mobile anions. This observation is in accordance with the transport function of potassium.

Correlation and linear regression functions have been calculated in order to investigate whether there would be any connection between the K^+ content and any separate anion content. The results of these calculations are given in Table 8. The low values of correlation coefficients show that there is no connection between the amount of potassium and any particular anion. Exeptions are formed by the high correlation coefficients between K^+ and NO_3^- especially in the high nitrogen plants and the negative correlation between K^+ and $H_2PO_4^-$ in the petioles in both nitrogen treatments.

This correlation between K^+ and NO_3^- can be caused by a simultaneous transport to the foliar system. The negative correlation between K^+ and $H_2PO_4^-$ is probably caused by pseudo correlation between $H_2PO_4^-$ and NO_3^-. The K^+ and NO_3^- data are shown in Figure 13.

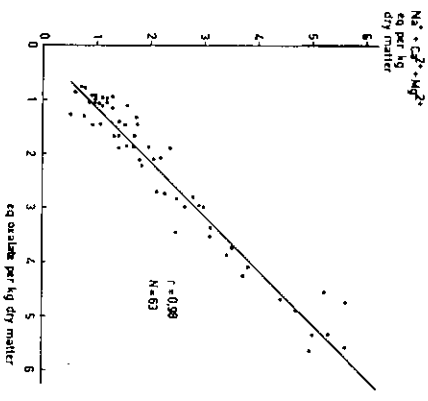

Figure 12. Relation between sum of $Na^+ + Ca^{++} + Mg^{2+}$ content and oxalate content in leaf blades and petioles of 45 days old sugar-beet plants.

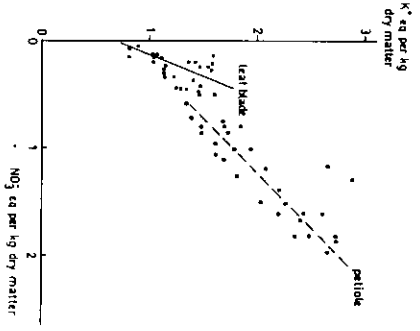

Figure 13. Relation between K^+ and NO_3^- content in leaf blades and petioles of 45 days old sugar-beet plants.

Table 8. Compilation of regression functions between K^+ and the anions ($y = a + bx$)

Fertilizer dose	250 kg N/ha			60 kg N/ha		
Number of samples	meq per kg d.m.	meq per kg d.m.	Correlation coefficient	Number of samples	meq per kg d.m.	meq per kg d.m.	Correlation coefficient
16 Lb	$K^+ =$ 320 +	12.26 (Cl^-)	0.78	15 Lb	$K^+ =$ 1570 −	8.27 (Cl^-)	−0.46
15 p	$K^+ =$ 1170 +	0.58 (Cl^-)	0.17	14 p	$K^+ =$ 1040 +	0.97 (Cl^-)	0.82
16 Lb	$K^+ =$ 730 +	1.45 (NO_3^-)	0.91	15 Lb	$K^+ =$ 700 +	3.36 (NO_3^-)	0.82
15 p	$K^+ =$ 560 +	1.08 (NO_3^-)	0.98	14 p	$K^+ =$ 1040 +	0.97 (NO_3^-)	0.82
16 Lb	$K^+ =$ 1250 −	0.30 (SO_4^{2-})	−0.24	15 Lb	$K^+ =$ 620 +	10.06 (SO_4^{2-})	0.29
15 p	$K^+ =$ 2070 −	2.15 (SO_4^{2-})	−0.06	14 p	$K^+ =$ 1510 +	10.66 (SO_4^{2-})	0.78
16 Lb	$K^+ =$ 1400 −	1.33 ($H_2PO_4^-$)	−0.61	15 Lb	$K^+ =$ 1550 −	1.60 ($H_2PO_4^-$)	−0.50
15 p	$K^+ =$ 3630 −	13.73 ($H_2PO_4^-$)	−0.92	14 p	$K^+ =$ 3050 −	7.88 ($H_2PO_4^-$)	−0.91
16 Lb	$K^+ =$ 740 +	1.02 [Σ(Carb-Ox)]	0.74	15 Lb	$K^+ =$ 870 +	0.96 [Σ(Carb-Ox)]	0.77
15 p	$K^+ =$ 760 +	1.51 [Σ(Carb-Ox)]	0.60	14 p	$K^+ =$ 1920 +	0.25 [Σ(Carb-Ox)]	0.12

Lb = Leafblade p = petiole

4. *The sodium-potassium substitution in regard to the accumulation of the inorganic cations in the foliar system*

All results so far obtained, indicate that when NO_3^- is in ample supply, the sugar-beet plant absorbs the cations and anions in equal quantities. Cations and anions are brought up through the xylem into the foliar system of the plant where the reduction of nitrate takes place. During this process the NO_3^- ion is substituted by the carboxyl-ion, which is here mainly oxalate. Together with the oxalate the cations Na^+, Ca^{2+}, Mg^{2+} and K^+ accumulate in the leaf tissue but at a different rate. Accumulation of Na, Ca and Mg in the aerial parts of the plant is a phenomenon often described in literature. In case of Ca and Mg the poor (low) solubility of the oxalates may be the most obvious reason for accumulation in sugar-beet leaves. Accumulation of Na in the leaves of sugar-beets and some other species like Spinach e.o., may be caused by inclusion of Na into Ca-oxalate crystals or by formation of double salts. Na-oxalate itself is reasonably water-soluble but the solubility of the acid salt Na-bioxalate is only moderate. The oxalates of potassium have roughly the same solubility as the sodium oxalates.

When sufficient quantities of Na, Ca and Mg are present to precipitate the oxalate as it is formed, potassium remains in solution being than the only mobile cation present in large quantities. When the supply of Na, Ca and Mg is less than the amount of oxalate, a certain part of potassium may also be tied up as oxalate. Data of Table 6 show that accumulation of potassium has been observed in our experiment when a low dose of nitrate of lime was applied. This fact is in agreement with the assumption that sometimes the oxalate-ion plays a part in the accumulation of potassium.

Literature data presented in Table 9, given as example to illustrate the fact that application of extra doses of sodium increases the total amount of potassium in the root section of the sugar-beet crop, support the above view of the mechanism. Moreover, it appears from *Rosanow's* data that out of the total amount of potassium present in the

Table 9. Uptake and distribution of potassium (by sugar-beet) after increasing doses of sodium

Draycott et al. [14]

Treatment	cwt Na^+/acre	K^+ in washed beet kg/acre
Na 0	0	31.8
Na 1	1	32.7
Na 2	2	34.2
Na 3	3	34.7

Rosanow [15]

K dose	0 kg K_2O		100 kg K_2O		200 kg K_2O	
Treatment	Total K in crop	$\frac{\text{K in roots}}{\text{K in total crop}} \times 100$	Total K in crop	$\frac{\text{K in roots}}{\text{K in total crop}} \times 100$	Total K in crop	$\frac{\text{K in roots}}{\text{K in total crop}} \times 100$
Na_1 (K_{40} + KAS)*	234**	19.0%	250	19.3%	235	14.3%
Na_2 (K_{20} + KAS)			271	21.7%	293	21.2%
Na_3 (K_{40} + Chs)	225	21.4%	263	23.1%	248	17.2%
Na_4 (K_{20} + Chs)			267	23.6%	292	22.9%

* K_{20}, K_{40} = potash salt 20 and 40 percent
KAS = lime – ammonium nitrate
Chs = Chilean nitrate

** kg K per ha

plant, a relatively greater part is to be found in the roots, as and when the dose of sodium applied is increased.
It is known that substitution of a part of K by Na occurs in other crops too. According to the above conception, sodium would be able to tie up a considerable amount of carboxylates in the foliage and by doing so release especially the K-ions. These free and mobile K-ions are than translocated to the growth regions.

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Bibliography

1. *Wit, C.T. de, Dijkshoorn, W.,* and *Noggle, J.C.:* Ionic balance and growth of plants. Versl. Landbouwk. Onderz. *69*, 15 (1963).
2. *Ulrich, A.:* Metabolism of organic acids in excised barley roots as influenced by temperature, oxygen and salt concentration. Amer. Jour. Bot. *29*, 220–227 (1942).
3. *Pierce, E.C.,* and *Appleman, C.D.:* Role of ether soluble organic acids in the cation-anion balance in plants. Plant Physiology *18*, 224–238 (1943).
4. *Chouteau, J.:* Les équilibres acides-bases dans le tabac Paraguay. Annales de l'Institut expérimental de Berberac 3 (1960).
5. *Dijkshoorn, W.,* and *Lampe, J.E.M.:* The separation of organic acids from *Lolium Perenne* L. Jaarboek I.B.S. 135–139 (1962).
6. *Tull, H.D.W. van:* Organic salts in plants in relation to nutrition and growth. Dissertation Wageningen.
7. *Jungk, A.:* Einfluß von Ammonium- und Nitrat-Stickstoff auf das Kationen-Anionen-Gleichgewicht in Pflanzen und seine Beziehung zum Ertrag. Sonderheft Landw. Forschung *21*, 50–63 (1967).
8. *Bengtsson, B.L., Bosund, J.,* and *Hylmö, B.:* Mineral Salts and Oxalate Content in Spinach Leaves as a Function of Development Stage. Zeitschrift Pflanzenernährung Düngung und Bodenkunde *115*, 192–199 (1966).
9. *Freeman, G.G.:* Studies on potassium nutrition of plants. Part II. Some effects of potassium deficiency on the organic acids of leaves. J. Sci. Fd. Agric. *18*, 171 (1967).
10. *Coïc, Y., Lesaint, C.,* and *Le Roux, F.:* Effets de la nature ammoniacale ou nitrique de l'alimentation azotée et du changement de la nature de cette alimentation sur le métabolisme des anions et cations chez la tomate. Ann. Physiol. Vég. *4*, 117–125 (1962).
11. *Dijkshoorn, W., Lathwell, D.J.,* and *de Wit, C.T.,* Temporal changes in carboxylate content of ryegrass with stepwise change in nutrition. Pl. Soil *24*, 369–390 (1968).
12. *Houba, V.J.G., van Egmond, F.,* and *Wittich, E.M.:* Changes in production of organic nitrogen and carboxylates (C-A) in young sugar-beet plants grown in nutrient solutions of different nitrogen composition. Neth. J. Agric. Sci. *19*, 39–47 (1971).
13. *van Egmond, F.,* and *Houba, V.J.G.:* Production of carboxylates (C-A) in young sugar-beet plants grown in nutrient solution. Neth. J. Agric. Sci. *18*, 182–187 (1970).
14. *Draycott, A.P., Marsch, J.A.P.,* and *Tinker, P.B.H.:* Sodium and potassium relationships in sugar-beet. J. Agric. Sci. *74* (3), 567–573 (1970).
15. *Rosanow, M.:* Stikstof en kalibemesting van suikerbieten op veenkoloniale gronden. Versl. Landbouwk. Onderz. *67*, 8 (1961).

The Effect of the Potassium Status of Tomato Plants on the Transport of Organic Compounds to the Fruits

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Summary

1. The influence of potassium nutrition on the transport of labelled compounds from tomato leaves to the fruits was studied.
2. Generally, the flux of the ^{14}C labelled compounds from the leaves to the fruits and roots increased with time.
3. After 6 hours of $^{14}CO_2$ application, most of the assimilated ^{14}C was still found in the leaves, ⅓ in the stem and only small amounts in the fruits and roots. This was true for the ethanol-soluble and the insoluble fraction.
4. Plants with a higher nutritional potassium status showed higher translocation rates for ^{14}C labelled compounds from the leaves to the fruits than plants poor in potassium.
5. Plants rich in potassium showed 18 hours after having finished the $^{14}CO_2$ application about 20% of the total label in the fruit whereas the plants poor in potassium had only accumulated about 8% of the total label in their fruits. According to this better translocation of organic material to the fruits the decrease of the label in leaves of potassium poor plants was less than in leaves high on potassium.
6. The sugar fraction of the ethanol-soluble extract showed the heaviest labelling, both in stems and fruits. Organic acids and amino acids were only slightly labelled.
7. Potassium was the predominant cation in all plant organs except the leaves. In the fruits, potassium accounted for 82% of total cation content.

It is well known that crop plants being adequately supplied with potassium accumulate large amounts of carbohydrate, sugar or fat in their storage tissues *(Forster* [3], *Herlihy* and *Carroll* [7], *Hofmann* and *von Schmeling* [8], *Mengel* and *Forster* [10]).

This favorable effect can be ascribed to a higher photosynthetic activity of plants better supplied with potassium. But also the rate of transport of organic material to the fruits and storage organs may be the cause for higher carbohydrate or sugar contents in these tissues. In this respect, investigations of *Hartt* [4, 5] are of interest revealing that particularly the K status of the plants considerably affects the transport of organic material in the phloem. *Dijkshoorn* [2] also assumes that K has an important function in the phloem transport by balancing the organic anions (carboxylates). The proper function of K in the phloem transport, however, remains still to be clarified. *Bowling* [1] found K concentrations in the sieve elements of *Vitis vinifera* up to 90 m*M*. He presumes that K is in some way involved in the phloem transport.

Hartt's [4, 5] experiments were carried out with sugar cane plants. As it is known that this plant species has a special CO_2 assimilation pathway *(Hatch* and *Slack* [6]), it was of interest to know whether the beneficial effect of a high K status on the translocation of organic material could also be observed in other plant species. The experiments described in this paper therefore were carried out with tomato plants. The study was focused on the question whether a high K status favors the transport of organic compounds from the leaves to the fruits. In order to eliminate differences in the photosynthetic activities due to the different K supply of the plants, the ^{14}C labelled material transported to the fruits was always related to the total labelled material of the whole plant.

Material and Methods

The experiments were carried out with tomato plants *(Solanum lycopersicum)*. They were grown in the greenhouse in solution culture of two different potassium levels: $K_1 = 1$ m*M* K, $K_2 = 10$ m*M* K, given as K_2SO_4. To reduce the plant material, the plants were cut above the first fruit branch and the number of fruits was reduced to 5.

The assimilation experiments were carried out when the first two fruits had reached a size of 3–4 cm in diameter. Then the plants were placed in a plexiglass growth chamber of about 600 l capacity, 6 or 8 plants per treatment, and they were fed with 1 mCi ^{14}C carbon dioxide, developed from barium ^{14}C carbonate with hydrochloric acid. The illumination was artificial, 10000 lux.

The plants were allowed to assimilate $^{14}CO_2$ for two hours at a constant temperature of 20 °C and a humidity of 90%; during the following 4 hours the residual $^{14}CO_2$ was pumped out of the box before this was opened and the first plants harvested (= time 0).

At harvesting the plants were divided into leaves, fruits, stems and roots. Each fraction was treated separately. The fresh material was extraced with 75% ethyl alcohol, the extract was evaporated to dryness *in vacuo* at 40 °C, the remainders were resolved with 50 ml water + 50 ml chloroform. The water phase was used for the subsequent experiments and for measurements in the liquid scintillation counter (Packard Tricarb Liquid Scintillation Spectrometer). The ethanol-insoluble plant material was dried, ground, combusted and absorbed in ethanol amine; the activity of this material was also measured in the liquid scintillation counter. By this technique it was possible to study the distribution of the ^{14}C labelled material in the whole plant. Further it was of interest to know which are the main assimilation products being transported to the fruits and which are the most important labelled storage compounds. For this purpose the extracts were fractionalized using ion exchangers. The aqueous solution was first passed through a cation exchanger (Dowex 50 W × 8, H^+form), thus separating the amino acids. The effluate from the cation exchanger was transferred to an anion exchanger in order to separate the anions from the sugars (Dowex 1 × 8, formiate form).

The effluate from this column contained the sugar fraction. The amino acids were eluted from the cation exchanger with 2 *N* NH_4OH, the organic acids from the anion exchanger with 5 *N* formic acid. The activities of these three fractions were measured in the liquid scintillation counter.

Results

Since each plant differs from the other it appears reasonable to express the distribution of the labelled ^{14}C in % of the total labelled material of the plant. Thus higher or lower assimilation intensities of individual plants are eliminated to some extent. Two series of experiments were carried out: in the first series, also the green fruits were allowed to assimilate; in the second series, the fruits were kept in the dark by covering them with an aluminium sheet in order to block assimilation by the fruits. The first important problem was: how long does it take before there are measurable amounts of labelled ^{14}C in the fruits. The answer can be found in Tables 1 and 2 showing the labelling of the various plant organs in dependance on time. From Table 1 it can be seen that immediately after application about one half of the labelled soluble assimilation products are to be found in the leaves, one third in the stems, and 10% each in the fruits and roots. 2 hours later, the

Table 1. Course of ^{14}C labelling in various plant organs in dependance on time (in % of total ^{14}C in the whole plant). Mean values of 2 plants each. K = 10 m*M*. Soluble fraction.

Hours after application	Leaves	Fruits	Stems	Roots
0 h	49	10	31	10
2 h	48	11	27	14
4 h	42	14	31	13
6 h	31	28	23	17

Table 2. Course of ^{14}C labelling in various plant organs in dependance on time (in % of total ^{14}C in the whole plant). Mean values of 2 plants each. K = 10 m*M*. Insoluble fraction.

Hours after application	Leaves	Fruits	Stems	Roots
0 h	77	2.6	18	2.6
2 h	74	4.4	17	4.1
4 h	74	4.9	16	4.8
6 h	63	16.0	15	5.3

amount of labelled products in the leaves and stems shows a slight decrease while in fruits and roots a slight increase was observed due to the transport of organic material from the leaves and stems to the fruits and roots. This trend becomes even more evident 6 hours after application. Then about only one third of the labelled soluble assimilation products could be found in the leaves; the amount of labelled ^{14}C in the fruits and roots had increased considerably. The same tendency, *i.e.* a decrease of labelled ^{14}C in the leaves, can also be seen in the insoluble fraction (Table 2). Immediately after application, 77% of the label is found in the leaves, 6 hours later it is only 63%. Also the amount of insoluble assimilation products increases in the fruits (from 2.6% at time 0 to 16% 6 hours later) and in the roots (2.6% at time 0, 5.3% 6 hours later). Both in the soluble and the insoluble fraction, there is only a slight decrease to be seen in the percentage amount of the labelled ^{14}C compounds in the stems, the tissues of which serve as main transport organs. In Tables 1 and 2 only one variable has been considered: the time after application. The main object of this study, however, was to examine the influence of varied K nutrition on the transport of assimilation products. Tables 1 and 2 show that there are already considerable amounts of labelled compounds accumulated in all plant organs after an application period of 6 hours. It should be stressed that the first plants, at zero time, had been harvested 6 hours after introducing $^{14}CO_2$ into the growth chamber. It therefore seemed reasonable to harvest in the following experiments 2 plants of each K level immediately after discontinuance of $^{14}CO_2$ application, whereas two plants were harvested 2 hours later.

Table 3 presents the percentage distribution of the ^{14}C labelled soluble assimilation products in tomatoes at two different K levels: $K_1 = 1$ m*M* K, $K_2 = 10$ m*M* K. At both K

Table 3. Influence of the K status on the distribution of ^{14}C labelled material in various plant organs. Soluble fraction; total label of the whole plant = 100%. Mean values of 2 plants each.

Hours after $^{14}CO_2$ application	Leaves		Fruits		Stems		Roots	
	K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2
0 h	58%	54%	2.8%	6.5%	36%	38%	2.4%	2.2%
2 h	52%	50%	5.9%	15.2%	39%	33%	3.3%	2.6%

levels immediately after application more than 50% of the total label was found in the leaves, about one third in the stems, whereas the amounts found in fruits and roots were considerably lower. It was interesting to see that more ^{14}C labelled compounds had accumulated in the fruits of the K_2-plants than in the fruits of the K_1-plants, whereas in the leaves rather the opposite pattern was observed. 2 hours later, the amount of the label in the leaves shows a light decrease at both K levels. In the stems no marked changes occurred. An increase of labelled assimilation products could be observed in the roots and especially in the fruits. It could be noted that the increase of radioactivity in the fruits of the K_2-plants is considerably higher than in the fruits of the K_1-plants.

Table 4 presents the data for the insoluble material of the same plants. The K status had no influence on the percentage proportion of the label in the leaves, stems and roots, but the fruits of the K_2-plants show a by 3 times heavier labelling. The percentage proportion of labelled compounds in the fruits compared with the total labelling of an individual plant can differ widely.

These differences are primarily due to the assimilation capacity of the fruits. Green fruits assimilate fairly high amounts of CO_2 while fruits at a more advanced stage of maturity have a low assimilation rate. In experiments with young green fruits not described in this paper, up to 30% of the total label were present in the fruits. This clearly demonstrates the high assimilation capacity of young green fruits.

In order to eliminate this CO_2 assimilation of green fruits, in a further experiment the young fruits were covered by aluminium sheets. By this technique, it was possible to measure the proper amounts of labelled compounds having been transported to the fruits. The results of this experiment are presented in Table 5. The amount of ^{14}C found in the fruits was very low immediately after application. In the K_1-plants only 0.2% of the total label was found in the fruits whereas in the K_2-plants 5.4% of the total label was found in the fruits. Since the amount of labelled compounds in the fruits was rather low, it was decided to harvest the next plants 18 instead of 2 hours later as before, in order to evaluate the influence of time on the transport of labelled assimilation products at different K levels. It was interesting to see that 18 hours later the amount of ^{14}C in the leaves had decreased to a larger extent in the K_2-plants than in the K_1-plants; in the stems no major

Table 4. Influence of the K status on the distribution of ^{14}C labelled material in various plant organs. Insoluble fraction; total label of the whole plant = 100%. Mean values of 2 plants each.

Hours after $^{14}CO_2$ application	Leaves		Fruits		Stems		Roots	
	K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2
0 h	70%	68%	0.7%	2.7%	27%	26%	1.4%	1.7%
2 h	71%	70%	1.2%	3.0%	27%	27%	1.3%	1.2%

Table 5. Influence of the K status on the distribution of ^{14}C labelled material in various plant organs when assimilation by the fruits was blocked. Soluble fraction. The values given are mean values of 6 plants each.

Hours after $^{14}CO_2$ application	Leaves		Fruits		Stems		Roots	
	K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2
0 h	65%	64%	0.2%	5.4%	33%	31%	0.3%	0.6%
18 h	52%	44%	7.7%	21.0%	32%	25%	4.7%	9.9%

Table 6. Percentage proportion of label in the water soluble sugar, amino and organic acid fractions. Total label of these 3 fractions = 100%

	Sugars		Amino acids		Organic acids	
	K_1	K_2	K_1	K_2	K_1	K_2
Fruits	86%	91%	5.5%	4.0%	8.2%	5.3%
Stems	89%	90%	5.6%	5.3%	5.7%	4.7%

changes in the ^{14}C label were observed. At both K levels the amount of ^{14}C found in the roots and fruits had increased within 18 hours. This increase was much higher in the fruits of the K_2-plants (21% of the total label) than in the fruits of the K_1-plants (7.7%). The same applies to the roots. Table 6 shows that by far the greatest part of the label is found in the sugar fraction, both in stems and fruits.

The influence of the K supply of the plants is not yet fully clarified, but it could be seen that generally the K_1-plants had a higher label in the fraction of amino acids and organic acids, whereas the K_2-plants showed a slightly higher label in the sugar fraction.

The percentage proportions of the different cations found in the various plant organs, expressed in *me* Ca, Mg, K and Na, are presented in Figure 1. The important part of K played in tomato fruits becomes obvious; only in the leaves the percentage amount of Ca is higher than that of K, in all other plant organs K is the dominating cation.

Discussion

The data of Tables 1 and 2 show that the ^{14}C labelled compounds are transported from the leaves to the fruits and roots. This is in full agreement with the transport conditions assumed for organic material in entire plants. The results clearly demonstrate that the major CO_2 assimilation organs are the leaves whereas fruits and roots are physiological sinks for these synthesised compounds. They further prove that the technique used here is suited to study the transport of organic compounds to the fruits of tomato plants. The transport rate depends on the K status of the plants, as can be seen from Tables 3, 4 and 5.

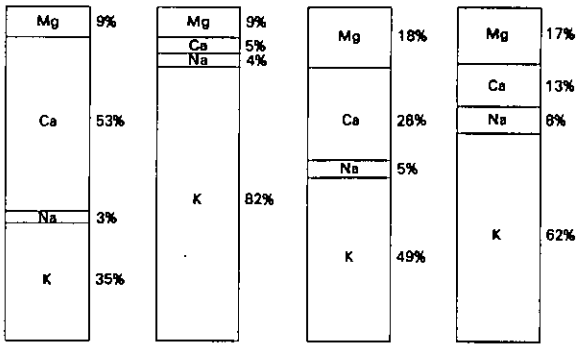

Figure 1. Percentage proprotions of K^+, Na^+, Ca^{++} and Mg^{++} in various plant organs expressed in *me*. ($K^+ + Na^+ + Ca^{++} + Mg^{++} = 100\%$)

Table 7. K content in dry matter (%)

Leaves		Fruits		Stems		Roots	
K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2
2.5	4.9	3.2	3.6	1.9	3.4	2.5	5.8

It is obvious that the plants with a higher K status accumulated higher proportions of ^{14}C labelled compounds in their fruits than those with a lower K status. This effect cannot be explained by a higher photosynthetic activity of the K_2-plants as the labelled quantities are expressed in relative values. The values obtained for the absolute ^{14}C assimilation rates, not discussed here, showed that the K nutrition had a major influence on the ^{14}C assimilation rate. This is in full agreement with observations of *Hartt* [*4, 5*]. There is no doubt that in plants poor in K CO_2 assimilation *(Jones* [*9*], *Peaslee* and *Moss* [*13*]*)* and also photophosphorylation are lowered *(Pflüger* [*14*]*)*. The K_1-plants used in the experiments here, however, were not so poor in K, as can be seen from Table 7. It therefore seems that the transport of organic compounds depends more on the K supply of the plants than photosynthesis.

Fruit development, particularly during the last phase, depends on the transport of organic material from the leaves to the fruits because at this stage the photosynthetic activity of the fruits themselves is rather low.

The data presented here cannot give major information concerning the transport mechanism of organic material. It is without any doubt that this transport chiefly takes place through the sieve elements of the phloem *(Milthorpe* and *Moorby* [*12*]*)*. Since K by far ranks first of all cations in the phloem sap, it is probable that it is in some way involved in the transport of organic material in the phloem or in the secretion of organic compounds into the sieve elements. *Dijkshoorn's* [*2*] opinion that K^+ has to balance the organic anions of the phloem sap cannot be proved by the data presented here. But it appears from Table 6 that the bulk of the labelled material being transported to the fruits are sugars and not organic anions.

The relatively high K content of the phloem sap seems to be responsible for the very high K proportion in the fruits (Figure 1). This indirectly demonstrates that the fruits mainly are supplied by the phloem sap which usually is rather poor in Ca and Na. The leaves show quite another cation pattern (Figure 1). Here the Ca contributes to more than 50% to the total cation equivalent. This high Ca content is mainly due to the Ca transported by transpiration via the xylem to the leaves *(Michael* and *Marschner* [*11*]*)*.

The high K requirements of the fruits are to some extent supplied by other plant organs. As can be seen from Table 7, the K content in the fruits of the two K treatments did not differ widely, whereas the K contents of the other plant organs were significantly affected by the K concentration of the nutrient solution.

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Bibliography

1. *Bowling, D.J.F.:* Evidence for the electroosmosis theory of transport in the phloem. Biochim. biophys. Acta *183*, 230–232 (1969).
2. *Dijkshoorn, W.:* The relation of growth to the ionic constituents of the plants. in: Rorison, J.H.: Ecological aspects of the mineral nutrition of plants. Blackwell Scientific Publications, Oxford 1969.
3. *Forster, H.:* Die Auswirkungen verschiedener variierter Ernährungsbedingungen auf den Ertrag und die Qualität der Zuckerrübe. Zucker *23*, 343–346 (1970).
4. *Hartt, C.E.:* Effect of potassium deficiency upon translocation of ^{14}C in detached blades of sugarcane. Plant Physiol. *45*, 183–187 (1970).
5. *Hartt, C.E.:* Effect of potassium deficiency upon translocation of ^{14}C in attached blades and entire plants of sugarcane. Plant Physiol. *44*, 1461–1469 (1969).
6. *Hatch, M.D.*, and *Slack, C.R.:* Photosynthetic CO_2-fixation pathways. Ann. Rev. Plant Physiol. *21*, 141–162 (1970).
7. *Herlihy, M.*, and *Carroll, P.J.:* Effects of N, P and K and their interactions on yield, tuber blight and quality of potatoes. J. Sci. Food Agric. *20*, 513–517 (1969).
8. *Hofmann, E.*, and *von Schmeling, B.:* Der Einfluß der K-Düngung auf Ertrag und Qualität des Sommerroggens. Z. Pflanzenbau und Pflanzenschutz, No. 4, 1–7 (1955).
9. *Jones, L.H.:* Carbon-14 studies of intermediary metabolism in potassium – deficient tomato plants. Canad. J. Bot. *44*, 297–307 (1966).
10. *Mengel, K.*, and *Forster, H.:* Der Einfluß der K-Konzentration der Nährlösung auf die Ertragsbildung, die Qualität und den K-Aufnahmeverlauf bei Hafer. Plant a. Soil *34*, 65–75 (1971).
11. *Michael, G.*, and *Marschner, H.:* Einfluß unterschiedlicher Luftfeuchtigkeit und Transpiration auf Mineralstoffaufnahme und -verteilung. Z. Pflanzenernähr., Düngg., Bodenkunde *96*, 200–212 (1962).
12. *Milthorpe, F.L.*, and *Moorby, J.:* Vascular transport and its significance in plant growth. Ann. Rev. Plant Physiol. *20*, 117–138 (1969).
13. *Peaslee, D.E.*, and *Moss, D.N.:* Photosynthesis in K- and Mg-deficient maize leaves. Soil Sci. Soc. Amer. Proc. *30*, 220–223 (1966).
14. *Pflüger, R.:* The biochemical way to higher yields keeping in mind the effects of fertilization methods. Lecture held at the 9th Congress of the International Potash Institute, Antibes, Fra

Influence of Potassium on Water Economy

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Summary

The role of potassium in the water economy of plants, including its effect upon transpiration coefficients, root pressure and osmotic pressure in plant sap in connection to transpiration and stomatal opening is discussed on the basis of the osmoregulating action of this element.

Most of the work done on potassium water relationship in cultivated plants reveals the fact, that plants well supplied with potassium exhibit lower transpiration coefficients than potassium deficient plants. This fact, well known since long time *(Arland [2])*, has been confirmed many times by recent results *(Blanchet et al. [4]; Amberger [1]; Kühn* and *Linser [17]; Linser* and *Herwig [18])*.

A new experiment in this field was conducted to comprehend the water saving effect of potassium not only by the final yield, but also by following the increments of growth ocurring during the successive stages of the vegetation period *(Herwig [10])*. The trials well supplied with potassium showed lower transpiration coefficients during the period of most intensive growth compared with the trials of lower potassium supply. On the other hand, there was no difference in the transpiration coefficients between both treatments, when the calculation was made on a final yield basis (Table 1). However, mention has to be made, that the control used in this experiment, contrary to the minus potassium control usually used, was supplied with a potassium level (0.42 g K_2O/pot) high enough to prevent visual K deficiency symptoms. This might explain the 10% difference in yield between the treatments reached in this experiment only.

With these results a basic question has been raised whether the lower values of transpiration coefficients are resulting from higher yields and unaltered water consumption or from

Table 1. Influence of K on the transpiration coefficient of oat (*Herwig*, 1970; unpublished data)

Growing period	K_1 yield (g)	water consum (ml)	transp. coeffic.	K_2 yield (g)	water consum (ml)	transp. coeffic.
4.6.–19.6.	21.6	6435	298	28.8	8042	279
19.6.–27.6.	16.8	4893	291	20.3	5309	262
27.6.– 3.7.	10.8	4717	437	10.1	5145	509
22.5.–31.7.	75.0	26742	393	84.1	30269	393
	(36.9 grains)		725	(40.9 grains)		740
	(38.1 straw)		702	(43.2 straw)		701

K_1 = 0.42 g K_2O/pot. K_2 = 2.0 g K_2O/pot. Water supply = 80% water capacity

unaltered yields and reduced water consumption. According to this a water saving effect can be achieved on quite different ways, and looking for the physiological reasons one must be aware of the different effects of potassium on the water economy.

Among these considerations the positive effect of potassium on the metabolism of carbohydrates and proteins may be recognized *(Amberger [1])*. On the other side the important role of potassium in osmoregulation and its responsibility for the establishment of the osmotic gradient between plant and soil has to be kept in mind. Such osmoregulating role of potassium is mainly maintained by the preferential uptake and continuous accumulation of potassium in the plant tissues, in spite of its usually lower concentration in the soil solution compared to that of other cations.

This regulating effect becomes evident in arid climate with high contents of sodium chloride in soil solution *(Di Giorgi et. al. [5])*. Citrus seedlings, grown under saline conditions varying from 13 to 35 mg Na/l, always take up more potassium than sodium. Even at the K:Na ratio of 1:30 in the culture solution, the potassium uptake was twice that of sodium. This leads to an osmoregulating effect, and balances the unphysiological Na:K ratio of the saline nutrient medium (Table 2). In experiments with a halophytic chlamydomonas, also the absorption rate of potassium rises when the NaCl concentration of the culture solution is raised *(van Auken* and *McNulty [23])*. This produces a 30to 40-fold increase of the K-concentration inside the cells compared to that of the growing medium, whereas the Na concentration reaches only 10 to 16% of that outside the cell (Table 3).

Following an effective osmoregulation in the roots increased values of osmotic pressure are detected in the different plant parts as well. This results from an active secretion of ions into the xylem, by which the osmotic pressure is transmitted to the vascular and other tissues of plant. Such phenomenon was reconfirmed by measuring the osmotic pressure of exudates, which always exhibit higher values than the nutrient medium *(Höfner* and *Herwig [12])*. Under normal conditions potassium is mainly responsible for the changes in osmotic pressure in the plant. However, such role of potassium can be taken over by other osmotically active organic and inorganic substances, when they become the major constituents in the medium and can be taken up by the plants *(Bernstein [3])*.

Table 2. K- and Na-content in Citrus aurantium (in % of the total content of K, Na, Ca and Mg in meq) as affected by salinity and K:Na ratio of the nutrient media (*Di Giorgi et al.* [1967])

Salinity of the culture solution	$\frac{K}{Na} \cdot 100$ in the culture solution									
(meq/l Na)	Control		3		20		30		50	
	K	Na	K	Na	K	Na	K	Na	K	Na
13.0	14.1	7.5	14.8	7.6	18.5	9.4	19.5	7.2	19.5	8.6
21.7	14.1	7.5	15.2	8.9	16.8	6.6	20.7	10.7	22.1	10.7
35.0	14.1	7.5	17.6	9.4	35.4	17.5	28.2	11.1	30.0	11.3

Table 3. Intracellular concentrations of sodium and potassium in Chlamydomonas *(Van Auken* and *McNulty* [1969])

Nutrient medium	Intracellular concentrations of	
	K	Na
1.0 mol NaCl 0.0025″ KCl	70 mmol	100 mmol
5.0 mol NaCl 0.0025″ KCl	110 mmol	800 mmol

The latter observation was proofed with experiments on root pressure. The promotion effect of potassium on the root pressure measured in terms of stem exudate can be replaced e.g. by Na^+, Rb^+, Mn^{++} and NO_3^- as shown by *Höfner* [*11*]; *Höfner* and *Herwig* [*12*] with sunflower plants and by *Mengel* and *Pflüger* [*21*] with corn seedlings, in which the root pressure accounts for 75% of the water transport *(Locher* and *Brouwer* [*20*]*)*. These experiments demonstrate the close relationship between establishment of an osmotic gradient between inner and outer solution and the active uptake of ions. Moreover, any promotion of the ion uptake through aeration or higher temperature of the culture solution or better nutrient supply may effect indirectly the regulation of the water economy of the plant. As mentioned before, the preferential uptake of potassium creates a higher concentration of this element and in turn increases osmotic pressure in the leaf (figure 1), thereby leading to a higher water retention against transpiration. It is interesting to see, that a detectable rise of the osmotic pressure in the sap of aerial plant parts during day time parallels an enhanced K transport to the same parts, while during night the reverse procedure occurs *(Bernstein* [*3*]*)*. Among the different elements in the leaf sap of beans, only K shows these changes of concentration, which parallels the diurnal fluctuation of osmotic pressure, while none of the other main elements shows a comparable behaviour. The reason for this special behaviour of potassium is not yet clear, but might be due to a preferential secretion into the phloem during night, which in turn accounts for a dominant concentration in the phloem sap and a basipetal translocation.

The previous observations might have a connection to a direct influence of potassium on the regulating mechanism of stomatal opening, which already has been mentioned 50 years ago *(Iljin* [*15*]*; Imamura* [*16*]*)*, and recently gained interest again by qualitative and quantitative experiments with epidermal strips *(Fujino* [*9*]*; Fischer* [*6, 7*]*; Fischer* and *Hsiao* [*8*]*; Humble* and *Hsiao* [*14*]*)*. According to these results potassium is taken up actively in light by the guard cells of the stomata and produces through a rise of cell turgor an optimal aperture of the stomata. In the dark, the guard cells release potassium again, which was demonstrated by ^{86}Rb, and this is thought to be responsible for a lowering of turgor and a subsequent closing of stomata. The other alkaline ions, with the exception of Rb, normally not present in plants, do not show this effect at comparable concentrations in light. Only at concentration of 100 m*M* in the buffered solution, serving as incubating medium for the epidermal strips, Na, Li, and Cs produce an opening of stomata similar to that of K at 0.1 m*M* concentration (figure 2). In the dark no difference in the degree of

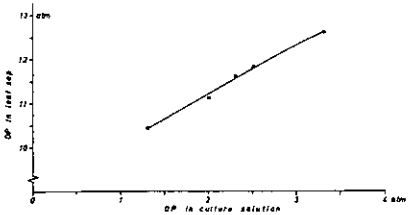

Figure 1. Osmotic pressure of leaf sap of dwarf red kidney beans as influenced by the osmotic pressure of the culture solution *(Lagerwerf* and *Eagle* [*1961*]*)*

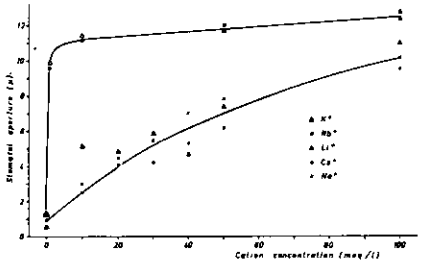

Figure 2. Stomatal opening in response to the cations K^+, Rb^+, Cs^+, Li^+, and Na^+, with Cl^- as the associated anion, in light *(Humble* and *Hsiao* [*1969*]*)*

stomatal opening is obtained by the different alkaline ions at equal concentration. The special effect of potassium is explained by a light dependent specific uptake of potassium *(Fischer* and *Hsiao* [*18*]*)*. This would be the first time, that a specific effect of potassium in the plant tissue is demonstrated.

Mention has to be made that the increase in potassium in the guard cells is accompanied by a decrease in starch content. Starch hydrolysis and increasing osmotic pressure due to sugar production have been known as a major reason for regulating stomatal opening since long time *(Iljin* [*15*]*)*, and it is questionable, which of these reactions plays the main role.

According to theoretical calculations, the potassium concentration inside the guard cells is increased very quickly to a threefold of the original value by the active uptake process in light. This would be quite sufficient to account for the necessary turgor, and there would be no need for an additional increase of osmotic pressure by starch hydrolysis *(Zelitsch* [*25*]*)*. In addition the pronounced stomatal opening after treatment with KCl compared with that of K_2SO_4 points to a secondary role of starch hydrolysis, because Cl^- is knwon to reduce the activity of hydrolytic enzymes *(Hofmann* [*13*]*)*. This might not exclude that starch hydrolysis is an integral part of an energy supply reaction for active ion uptake, through which the guard cells, lacking any plasmodesmatal connection to the surrounding cells, can only maintain a high ion content *(Fischer* and *Hsiao* [*8*]*)*.

A different action of the accompanying anion on this potassium effect can be observed with Cl^- and SO_4^- (Figure 3) though 70% of the light dependent potassium uptake is independent of Cl^- uptake *(Pallaghi* [*22*]*)*. On the other side, there is a marked influence of Ca on this uptake, which can be seen in a higher sodium effect on stomatal opening compared to that of potassium at the presence of 50 m*M* Ca *(Pallaghi* [*22*]*)*. A higher effect of sodium on stomatal opening than potassium was also reported by *Willmer* and *Mansfield* [*22*]. The same authors drew attention to the importance of divalent cations and pH-values of the incubation medium, which contributes to the specific effect of the monovalent ions. However differences in the reaction of different plant species were

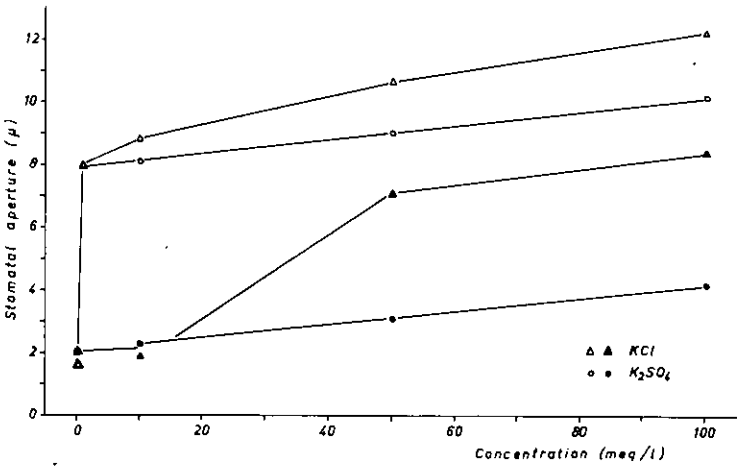

Figure 3. Effects on stomatal opening of the anions Cl^- and SO_4^{--} in light (open symbols) and darkness (closed symbols), when associated with K *(Humble* and *Hsiao* [*1969*]*)*

observed as could be shown in a comparative study with *Vicia* and *Commelina (Willmer* and *Mansfield [24]).*

Further confirmation and additional results are needed before a true evaluating of this potassium effect can be achieved. Related to water economy, this specific K-function would have to be considered direct and indirect at the same time, because on the one side it regulates transpiration, and on the other it affects CO_2-assimilation. But a confirmation of such function would present a valuable contribution to a better understanding of the observations, we till now only can declare as water saving effects of potassium.

Bibliography

1. *Amberger, A.:* Funktionen des Kalis in der Pflanze. Kalibriefe *10,* Fachgeb. 2, 1. Folge, 1–6 (1968).
2. *Arland, A.:* Kurzfristige Vorprüfung von Kulturmaßnahmen. Die Deutsche Landwirtschaft *4,* 13–16 (1950).
3. *Bernstein, L.:* Osmotic adjustment of plants to saline media. II. Dynamic phase. Amer. J. Bot. *50,* 360–370 (1963).
4. *Blanchet, R., Studer, R.,* and *Chaumont, C.:* Quelques aspects des interactions entre l'alimentation potassique et l'alimentation des plantes. Ann. agron. (Paris) *13,* 93–110 (1962).
5. *Di Giorgi, M.C., Fichera, P.,* and *Tropea, M.:* Nuovi orentamenti sull'utilizzazione delle acque saline. III. L'influenza del rapporto potassio-sodio su colture salinosensibili *(Citrus aurantium* L.), Agrochimica *11,* 167–175 (1967).
6. *Fischer, R.A.:* Stomatal opening: role of potassium uptake by guard cells. Science *160,* 784–785 (1968).
7. *Fischer, R.A.:* Stomatal opening in isolated epidermal strips of *Vicia faba.* I. Response to light and CO_2-free air. Plant Physiol. *43,* 1947–52 (1968).
8. *Fischer, R.A.,* and *Hsiao, T.C.:* Stomatal opening in isolated epidermal strips of *Vicia faba.* II. Response to KCl concentration and the role of potassium absorption. Plant Physiol. *43,* 1953–58 (1968).
9. *Fujino, M.:* Adenosinetriphosphate and adenosinetriphosphatase in stomatal movement. Sci. Bull. Fac. Educ. Nagasaki Univers. *18,* 1–47 (1967).
10. *Herwig, K.:* Unpublished data, 1970.
11. *Höfner, W.:* Biogene Komplexverbindungen als Transportformen aufgenommener Mikronährstoffe in der Pflanze. Habilitationsschrift, Gießen, 1967.
12. *Höfner, W.,* and *Herwig, K.:* Manganeinfluß auf Volumen und osmotischen Wert des Exsudats junger Sonnenblumen. Physiol. Plant *19,* 31–39 (1966).
13. *Hofmann, E.:* Das Sulfatproblem als Folge der veränderten Düngerwirtschaft. Bayer. landw. Jb. *34,* Sonderh. 1, 19–26 (1957).
14. *Humble, G.D.,* and *Hsiao, T.C.:* Specific requirement of potassium for light-activated opening of stomata in epidermal strips. Plant Physiol. *44,* 230–234 (1969).
15. *Iljin, W.S.:* Physiologischer Pflanzenschutz gegen schädliche Wirkung von Salzen. Biochem. Z. *132,* 526–542 (1922).
16. *Imamura, S.:* Untersuchungen über den Mechanismus der Turgorschwankungen der Spaltöffnungsschließzellen. Japan. J. Bot. *12,* 251–347 (1943).
17. *Kühn, H.,* and *Linser, H.:* Beziehungen zwischen Kaliumernährung, Wasserversorgung und Chlorcholinchlorid(CCC)-Wirkung bei Sommerweizen. Kalibriefe *8,* Fachgeb. 2, 1. Folge, 1–10 (1966).
18. *Linser, H.,* and *Herwig, K.:* Zusammenhänge zwischen Bewindung, Transpiration und Nährstofftransport bei Lein unter besonderer Berücksichtigung einer variierten Wassergabe und Kalidüngung. Kalibriefe *10,* Fachgeb. 2, 2. Folge, 1–12 (1968).
19. *Lagerwerf, J.V.,* and *Eagle, H.C.:* Osmotic and specific effects of excess salts on beans. Plant Physiol. *36,* 472–477 (1961).
20. *Locher, J.T.,* and *Brouwer, R.:* Preliminary data on the transport of water, potassium and nitrate in intact and bleeding maize plants. Medeling 238 van het J.B.S., Wageningen, 41–49 (1964).
21. *Mengel, K.,* and *Pflüger, R.:* Der Einfluß verschiedener Salze und verschiedener Inhibitoren auf den Wurzeldruck von *Zea mays.* Physiol. Plant. *22,* 840–849 (1969).
22. *Pallaghy, C.K.:* The effect of Ca^{++} on the ion specificity of stomatal opening in epidermal strips of *Vicia faba.* Z. Pflanzenphysiol. *62,* 58–62 (1970).
23. *Van Auken, O.W.,* and *McNulty, J.B.:* Intracellular concentrations of sodium, potassium and chloride in a halophytic chlamydomonas. Plant Physiol. *44,* Suppl. Nr. 92, 8–20 (1969).
24. *Willmer, C.M.,* and *Mansfield, T.A.:* A critical examination of the use of detached epidermis in studies of stomatal physiology. New Phytol. *68,* 363–375 (1969).
25. *Zelitch, J.:* Environmental and biochemical control of stomatal movement in leaves. Biol. Rev. *40,* 463–482 (1965).

Recent Aspects of the Influence of Potassium on Stomatal Opening and Closing

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Comprehension of the mechanism of stomatal opening and closing is not only of theoretical interest but also of practical value, because these processes control plant transpiration and uptake of CO_2 closely connected with plant growth and yield.
In a film, stomatal opening and closing was shown during the 2nd Session of this Colloquium, with the three main types of stomata *(Trolldenier [16])*. A comprehensive review of the function and different build-up of guard cells is given in another publication *(Trolldenier [17])*.
As already shown by *v. Mohl [9]*, stomatal opening is controlled by variations in turgor and volume of the guard cells.
Numerous hypotheses have been developed to find an explanation for the variations in turgor. At first, it was assumed that stomatal opening in light could be ascribed to the photosynthetic formation of osmotically effective sugars. Today it is known that the photosynthetic activity of guard cells is too poor for bringing about the observed rapid increase in osmotic pressure.
Recently, interest has focused on the part of inorganic ions, especially potassium, played in stomatal opening and closing. Further information on this subject will be given in the following.

Influence of various ions on stomatal opening and closing

Older papers, discussed in detail by *Stålfelt [13]*, mainly deal with the influence of salts on the enzymatic hydrolysis of guard cell starch. *Iljin [7]* was the first to find that potassium and sodium salts favour the hydrolysis of starch in the guard cells whereas calcium and magnesium salts retard it. According to *Iljin* and other researchers, hydrolysis of starch and formation of soluble sugars would result in an increase of osmotic pressure and thus be the virtual cause for stomatal opening. *Imamura [8]* was the first to ascribe a decisive part to the potassium ion in the function of stomata. According to *Imamura*, the potassium content of the guard cells rises during opening and is lowered when the stomata close again. This action of K was ascribed to a swelling of the plasma colloids.
It was not till recently that potassium has been recognized to have a direct influence on the variations in volume of guard cells. According to this new concept, it is assumed that the amount of K^+ and the associated anions taken up during opening would be responsible for the increase in turgor and volume of guard cells (e.g. *Fujino [4]*, *Fischer* and *Hsiao [2]*, *Humble* and *Hsiao [5]*, *Thomas [14]*).

Several workers, using different methods, found a higher potassium content in guard cells of opened stomata than in those of closed stomata. *Fujino* [4] as well as *Humble* and *Hsiao* [6] used histochemical staining of potassium, *Hsiao et al. (Fischer* and *Hsiao* [2], *Humble* and *Hsiao* [6]) measured the uptake of labelled rubidium as a tracer for potassium in guard cells of epidermal strips, and *Sawhney* and *Zelitch* [12] used the electron microprobe technique. All results obtained clearly demonstrate that guard cells of open stomata contain more potassium than those of closed stomata. Calculations of *Fischer* and *Hsiao* [2] indicated that K^+ concentration of guard cells in epidermal strips of *Vicia faba* appeared to be more than tripled. The initial K^+ concentration of closed stomata was 150 m*M* or slightly less. With the aid of the electron microprobe, *Sawhney* and *Zelitch* [12] found a potassium concentration of 210 m*M* in closed tobacco stomata. Nearly the same concentration was observed in adjacent epidermal cells, viz. 190 m*M*, and 500 m*M* in fully opened stomata. *Fischer* and *Hsiao* as well as *Sawhney* and *Zelitch* stated a direct relationship between potassium concentration in guard cells and stomatal aperture.

Evidence for a specific stimulating effect of potassium on stomatal opening in light has been provided particularly by investigations of epidermal strips floating on salt solutions. In isolated epidermal strips of *Vicia faba*, stimulation of stomatal opening by KCl was highest at about 10 m*M (Fischer* and *Hsiao* [2]). This is supported by very recent results obtained by *Thomas* [14] with epidermal strips of tobacco, using another technique. With a newly constructed solution-flow porometer, opening and closing of stomata of the same epidermal strip could continuously be recorded. In dependence on alternating light and dark the stomata of epidermal strips of tobacco bathed in 10 m*M* KCl opened in light and closed in the dark. *Thomas* also found a reduction in aperture under the influence of higher K^+ concentrations (20 and 50 m*M*) whereas *Hsiao et al.* even observed a maximum of stomatal aperture in *Vicia faba* up to a concentration of 100 m*M* K^+.

At a concentration of 10 m*M* KCl, solutions of other monovalent cations, such as Li^+, Cs^+ and Na^+, have very little influence and do not show any light-activated specifity *(Humble* and *Hsiao* [5]). Simultaneous recording of stomatal aperture and uptake of labelled rubidium and sodium, respectively, showed a light-dependent stimulation of opening together with active ion uptake only for rubidium and not for sodium *(Humble* and *Hsiao* [6]). No opening is produced by NH_4^+ and Mg^{++}. The effect of Ca^{++}, a cation often considered as being essential for maintaining the integrity of the cell wall, is an open question. In various papers, a reduction in stomatal aperture or entire closure of stomata after addition of Ca^{++} to the bathing medium is reported *(Fujino* [4], *Pallaghy* [10], *Willmer* and *Mansfield* [18]).

Some authors not only stated a light-dependent stomatal opening in the presence of potassium but also for sodium. *Pallaghy* [10], using epidermal strips of *Vicia faba*, observed nearly the same stomatal aperture with 10 m*M* NaCl as with an equimolar solution of KCl. Since the first measurement had been made after 2½ hours only, it cannot be excluded that differences existed at least in the aperture rate. *Willmer* and *Mansfield* [18], in contradiction to *Fujino* [4], also stated light-stimulated opening in *Commelina communis* with NaCl. Results obtained by *Thomas* [14], using the very sensible solution-flow porometer method, are more convincing. The response to a change from light to dark by stomata of epidermal strips of tobacco when bathed in 10 m*M* NaCl was considerably less than with 10 m*M* KCl. In contrast to the stomata bathed in KCl, those floating in a NaCl solution only gradually opened in the dark. When adding 1 m*M* KCl to the NaCl medium of this epidermal strip, the aperture immediately diminished. With a subsequent change from light to dark the stomata resumed their normal behaviour.

Thomas concluded from his experiments that the K^+ influx mechanism probably has only low affinity for Na^+.

There are, however, some plants, e.g. *Kalanchoe marmorata*, which apparently have no light-dependent K uptake mechanism. This Crassulacea is known for stomatal opening in the dark and closing in light. In epidermal strips, the stomata of *K. marmorata* showed no response in light nor in the dark when treated with 10 m*M* KCl. With 10 m*M* NaCl as bathing solution, however, the stomata opened in the dark and closed in light just as those of intact plants *(Thomas [14])*. Similarly to illumination, the addition of 0.1 m*M* ATP (adenosine triphosphate) resulted in a persistent diminution of aperture. According to *Thomas*, the behaviour of stomatal opening of *K. marmorata* may be explained by the fact that negative charges of the guard cells are built up in the dark due to the synthesis of organic acids thus leading to a diffusion of Na^+ in the guard cells. Illumination increases the supply of energy substrate (ATP) in the course of photosynthesis and stimulates a Na^+ efflux mechanism so that the stomata will close. These interesting findings need, however, confirmation by experiments with other plants whose stomata also open in the dark and close in light.

Whereas the influence of cations on the behaviour of guard cells differs rather widely, the associated anions seem to be of minor importance *(Humble* and *Hsiao [65], Thomas [14])*. Cl^-, Br^- and NO_3^-, in the form of potassium salts, showed the same reaction, SO_4 led to a somewhat smaller aperture only at concentrations of more than 10 me/l.

Hypotheses regarding the specific mechanism of potassium accumulation

The above mentioned results suggest that the accumulation of potassium in guard cells during the opening of stomata in light might be mediated by a light-stimulated K^+-specific mechanism, denoted also as 'potassium pump'.

The assumption of *Fujino [4]* that an ATPase system might be involved in the transport of K^+ has been supported by experiments of *Thomas [15]*. According to *Thomas*, the assumption that K^+ transport through the membrane of guard cells is effected by means of a carrier offers a good explanation for the high K^+ uptake rate during stomatal opening, being greater than that observed in other plant cells. The data of *Humble* and *Hsiao [6]* suggest that the photosystem is located in the chloroplasts of guard cells and the cyclic electron flow supplies the necessary energy for ATP synthesis. Until now, it could not yet be explained how potassium moves out of guard cells during stomatal closure. It can, however, be assumed that this is a passive process.

The importance for the adjustment of stomatal aperture to prevailing ecological conditions

The influence of potassium on stomatal aperture could also be clearly demonstrated in field experiments. Plants having received different applications of potassium show different capacity of aperture control. Plants adequately supplied with potassium, in comparison with K deficient plants, behave in warm summer days like plants resistent to drought against plants susceptible to drought. Stomata of well nourished plants open early in the morning and close when drought is to be feared. According to *Frommhold [3]*, the stomata of potassium-deficient plants are the most widely opened in the morning. They

remain open also during midday heat so that transpiration is much higher. An explanation for these remarkable findings could not yet be given.

According to *Cooper et al.* [*1*], heavy supplies of K also increase the number of stomata per unit leaf area. With sufficient water supply, stomatal aperture of lucerne, according to these authors, is increased with increasing potassium applications. The same has been observed in maize *(Peaslee* and *Moss* [*11*]*)*. Since gaseous exchange through widely opened stomata is much better, large potassium dressings thus exert a favourable influence on photosynthesis and, in the end, on yield.

Bibliography

1. *Cooper, R.B., Blaser, R.E.,* and *Brown, R.H.:* Potassium nutrition effects on net photosynthesis and morphology of alfalfa. Proc. Soil Sci. Soc. Am. *31*, 231–235 (1967).
2. *Fischer, R.A.,* and *Hsiao, T.C.:* Stomatal opening in isolated epidermal strips of Vicia faba. II. Responses to KCl concentration and the role of potassium absorption. Plant Physiol. *43*, 1953–1958 (1968).
3. *Frommhold, J.:* Untersuchungen über den Einfluß von Stickstoff- und Kaliummangel auf das Verhalten der Stomata von Hafer und Runkelrübe unter besonderer Berücksichtigung der Anwelktranspiration. Wiss. Z. Karl-Marx-Univ. Leipzig *15*, 83–94 (1966).
4. *Fujino, M.:* Role of adenosine triphosphate and adenosine triphosphatase in stomatal movement. Sci. Bull. Fac. Educ. Nagasaki Univ. *18*, 1–47 (1967).
5. *Humble, G.D.,* and *Hsiao, T.C.:* Specific requirement of potassium for light-activated opening of stomata in epidermal strips. Plant Physiol. *44*, 230–234 (1969).
6. *Humble, G.D.,* and *Hsiao, T.C.:* Light-dependent influx and efflux of potassium of guard cells during stomatal opening and closing. Plant Physiol. *46*, 483–487 (1970).
7. *Iljin, W.S.:* Wirkung der Kationen von Salzen auf den Zerfall und die Bildung von Stärke in der Pflanze. Biochem. Z. *132*, 494 (1922).
8. *Imamura, S.:* Untersuchungen über den Mechanismus der Turgor-Schwankung der Spaltöffnungsschließzellen. Jap. J. Bot. *12*, 251–346 (1943).
9. *Mohl, H., v.:* Welche Ursachen bewirken die Erweiterung und Verengung der Spaltöffnungen? Bot. Zeit. 1856.
10. *Pallaghy, C.K.:* Die Wirkung von Ca auf die Ionenspezifität der stomatären Öffnungsbewegung bei Epidermisstreifen von Vicia faba. Z. Pflanzenphysiol. *62*, 58–52 (1970).
11. *Peaslee, D.E.,* and *Moss, D.N.:* Photosynthesis in K- and Mg-deficient Maize (Zea mays L.) leaves. Proc. Soil Sci. Soc. Am. *30*, 220–223 (1966).
12. *Sawhney, B.L.,* and *Zelitsch, J.:* Direct determination of potassium ion accumulation in guard cells in relation to stomatal opening in light. Plant Physiol. *44*, 1350–1354 (1969).
13. *Stålfelt, M.G.:* Die stomatäre Transpiration und die Physiologie der Spaltöffnungen. Handb. Pflanzenphysiol. Vol. *3*, 351–426 (1956), Springer-Verlag Berlin.
14. *Thomas, D.A.:* The regulation of stomatal aperture in tobacco leaf epidermal strips I. The effect of ions. Aust. J. biol. Sci. *23*, 961–979 (1970).
15. *Thomas, D.A.:* The regulation of stomatal aperture in tobacco leaf epidermal strips. II. The effect of ouabain. Aus. J. biol. Sci. *23*, 981–989 (1970).
16. *Trolldenier, G.:* Bewegung der Spaltöffnungen. Film C 973. Inst. wiss. Film, Göttingen 1968.
17. *Trolldenier, G.:* Spaltöffnungen – lebenswichtige Organe der Pflanzen. Mikrokosmos H. 4, 117–126 (1968).
18. *Willmer, C.M.,* and *Mansfield, T.A.:* Active cation transport and stomatal opening: a. possible physiological role of sodium ions. Z. Pflanzenphysiol. *61*, 398–400 (1969).

An Assessment of Plant Nutrient Content as a Guide to Nutritional Status

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1. *Introduction*

The value of plant nutrient content as a guide to nutrient supply depends on the extent to which the plant content of a nutrient is independant of variation in external factors other than that nutrient. In our work, we have found that plant nutrient contents frequently were not well correlated with soil nutrient levels (Figure 1). It is the purpose of this paper to show how the low level of correlation could be due in part to variations in plant content which were independent of soil nutrient levels.

2. *Theory*

Several workers have shown that only the apical portions of the root are active in the uptake of nutrients. The rate per unit time at which a nutrient is taken up across unit area of active root surface depends on (i) the rate per unit time 'c' at which the nutrient is supplied from the medium and (ii) on a plant determined parameter 'a' which has been termed 'the plant demand coefficient' *(Nye* and *Tinker [1])*.

$$u = a\,c \quad \ldots\ldots\ldots (1)$$

Each segment of root is active for a short period (t) when it is near the apex. Over this period, when the total root surface area and the root surface area active in uptake (RSA) may be assumed constant, the total uptake (U) for this period (t) is determined by the rate of uptake per unit area and by the surface area of the number of segments active in uptake (RSA) and by the length of the period.

$$U = u\,(RSA)\,t \quad \ldots\ldots\ldots (2)$$

Combining (1) and (2) gives

$$U = ac\,(RSA)\,t \quad \ldots\ldots\ldots (3)$$

For longer periods of time (i.e. a growing season) although total root surface area is changing during the period, the final total root surface provides an estimate of the root surface which has been active during the period, each root segment being active only for a short period (t) when it is near the apex. Using final root surface area and an average value for a and c (the average of all the values of a and c occurring in the system during the season) then (3) becomes:

$$U = \bar{a}\bar{c}(RSA)t \quad \ldots\ldots\ldots (4)$$

Nutrient content (X) is generally expressed as a percentage of dry matter yield.

$$\%\,X = 100\frac{U}{\text{yield}} \quad \ldots\ldots\ldots (5)$$

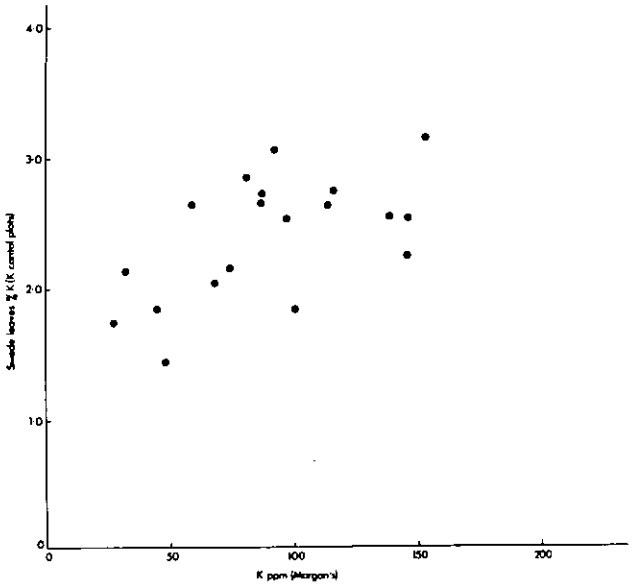

Figure 1a. Relation between % K in the plant and soil K.

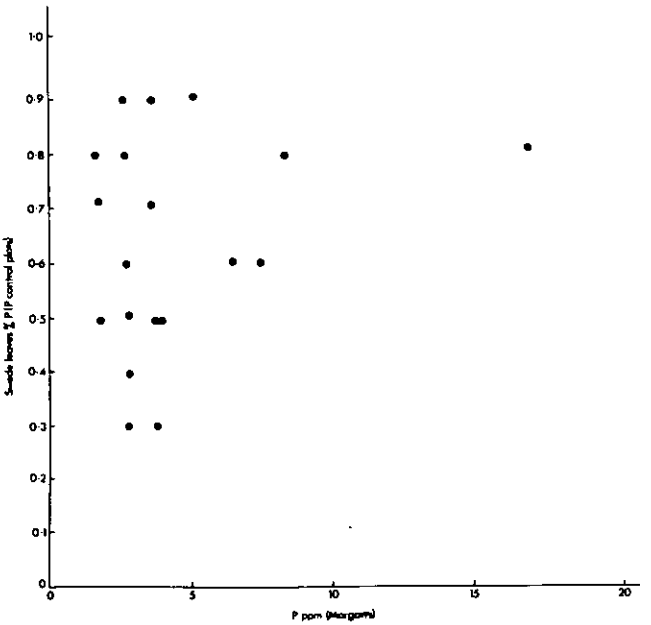

Figure 1b. Relation between % P in the plant and soil P.

Combining (4) and (5)

$$\%X = 100\bar{a}\bar{c}\frac{t(RSA)}{Yield} \quad \ldots\ldots\ldots\ldots (6)$$

From (6) it is clear that the percentage content of a nutrient in the plant will vary subject (Table 1) to fluctuations in (1) nutrient supply rate from the rooting medium (2) R.S.A./yield ratio and (3) the plant demand coefficient. *Brouwer* and *Troughton* have put forward, as a general rule, that the ratio root yield/total yield decreases as the supply of mineral nutrients to the plant increases and increases as light or CO_2 supply is increased (Troughton [2] and Brouwer [3]. It has been suggested that this decrease in the ratio operates as a mechanism for the maintenance of balance in the uptake of nutrients from the roots and of carbohydrate from the leaves. Therefore, if this suggestion is true, % nutrient at a given level of supply of that nutrient to the roots would vary as climatic conditions or other nutrients varied.

Table 1.

$\%K = 100\bar{a}_k\bar{c}_k t\frac{(RSA)}{Yield}$ $\%P = 100\bar{a}_p\bar{c}_p t\frac{(RSA)}{Yield}$ $\%N = 100\bar{a}_n\bar{c}_n t\frac{(RSA)}{Yield}$

3. *Experimental*

Swedes (Var. Danish Bangholm) were sown at 19 sites in the Irish Republic. The sites were selected to represent a wide range of the climate and soil conditions occurring in the country. The crop was sown in June in drills 0.711 m wide and thinned to approximately 50,000 plants per hectare in July. In one block at each site potassium was applied at 0, 80, 160 and 240 kg K per hectare (as muriate of potash, 50% K). The treatments were applied in a random block design with three replicates. In a second block phosphorus was applied at 0, 36, 72 and 112 kg P per hectare (as superphosphate, 8% P) using the same design as for the potassium experiment. A basal dressing of 60 kg N per hectare (as calcium ammonium nitrate, 26% N) was used in each experiment. In the potassium experiment a basal dressing of 92 kg P/ha (as superphosphate, 8% P) was applied. In the phosphorus experiment a basal dressing of 160 kg K per hectare (as muriate of potash, 50% K) was applied.

The plots measured 5.5 x 16.5 m. Two metres of the end of each plot were discarded. The yield data discussed in the following paragraphs refers to the material harvested from the four centre drills of each plot. The crop was harvested in October. Leaf samples for chemical analysis were taken in August.

4. *Results and Discussion*

4.1 *Potassium experiment*

The results of the potassium experiment are given in Tables 2 and 3. The data for sites giving similar responses to potassium have been averaged.

The ratio RSA/total yield was unaffected by potassium application within sites. The arithmetic increase in the rate of potassium application was not reflected in the increase in

Table 2. Potassium experiment. Yield data

% Response Category	Total Yield (mT/Ha)				Root/Leaf				RSA*/Total Yield				No. Sites
	K_0	K_1	K_2	K_3	K_0	K_1	K_2	K_3	K_0	K_1	K_2	K_3	
0– 3%	74.5	74.8	76.5	74.5	2.49	2.62	2.71	2.83	.068	.068	.069	.070	7
7–15%	59.0	66.9	66.7	64.6	1.70	1.84	1.97	1.98	.068	.069	.069	.070	7
18–26%	61.2	71.4	70.3	70.1	2.0	2.62	2.68	2.78	.072	.070	.071	.070	5

* Root surface area is calculated from the weight of swollen root. *Bleasedale [4]* has stated that root surface area of fibrous roots in swedes is proportional to (swollen root weight)^{66}. Our own observations have shown that the weight of fibrous roots is related to weight of swollen root in this way.

Table 3. Potassium experiment. Plant chemical composition

% Response Category	% K				% P				% N				No. Sites
	K_0	K_1	K_2	K_3	K_0	K_1	K_2	K_3	K_0	K_1	K_2	K_3	
0– 3%	2.3	2.6	2.8	2.7	.74	.74	.76	.74	6.9	6.6	6.7	6.9	7
7–15%	2.4	2.7	3.0	3.2	.81	.75	.69	.74	6.9	6.9	6.7	6.6	7
18–26%	2.3	2.6	2.7	2.8	.72	.74	.77	.75	6.3	6.4	6.4	6.4	5

% K in the plant in response to potassium application i.e. increase in % K tended to level off as potassium application increased. In terms of the equation for % K in Table 1 these results indicated that the plant demand coefficient for potassium decreased with increasing application of potassium i.e. RSA/yield was constant and $\bar{c}_k$ was increasing arithmetically – therefore the failure of % K to increase arithmetically was due to a decrease in $\bar{a}_k$. The relative constancy of both % P and % N within sites may be interpreted in terms of the equation for % P and % N in Table 1. RSA/yield and $\bar{c}_p$ and $\bar{c}_n$ were constant in both cases. The constancy of % N and % P when potassium was applied indicated that potassium applications did not affect the plant demand coefficient for either nitrogen or phosphorus. On the basis of the suggestions by *Troughton [2]* and *Brouwer [3]* the higher overall ratio of RSA/yield for the higher response group of sites may be taken to indicate a deficiency of nutrient or nutrients at these sites. The fact that % N values were lower in this group (Table 3) supports this indication.

4.2 *Phosphorus Experiment*

The phosphorus experiment results are given in Tables 4 and 5. The ratio RSA/yield was reduced by phosphorus application in all groups of sites. The effect was most marked in the high response group of sites (i.e. in the phosphorus deficient group of sites). As in the case of potassium, phosphorus was applied in arithmetically increasing increments. In general, the increase in % P (Table 5) in response to phosphorus applications tended to level off with increasing level of application of fertiliser. This levelling off can be interpreted in terms of the equation for % P (Table 1) as the result of the decreasing RSA/yield ratio or of a decrease in the plant demand coefficient for phosphorus or of both.

The decrease in % K (Table 5) with phosphorus applied reflects the decreasing ratio RSA/yield. The data provides no evidence to show that the plant demand coefficient for potassium changed as phosphorus applications increased. The % N values (Table 5) show

Table 4. Phosphorus experiment. Yield data

% Response Category	Total Yield (mT/Ha)				Root/Leaf				RSA/Total Yield (g/plant)				No. Sites
	P_0	P_1	P_2	P_3	P_0	P_1	P_2	P_3	P_0	P_1	P_2	P_3	
6– 8%	77.2	86.2	85.4	84.7	2.41	2.41	2.43	2.38	.067	.066	.065	.064	4
9–11%	63.8	67.6	70.5	70.3	2.91	2.78	2.65	2.74	.073	.071	.069	.071	5
13–20%	56.3	64.4	71.3	68.7	2.53	2.32	2.36	2.26	.077	.074	.070	.071	5
22–35%	41.5	60.7	63.6	69.2	2.63	2.30	2.33	2.26	.085	.071	.071	.069	5

Table 5. Phosphorus experiment. Plant chemical composition

% Response Category	% K				% P				% N				No. Sites
	P_0	P_1	P_2	P_3	P_0	P_1	P_2	P_3	P_0	P_1	P_2	P_3	
6– 8%	3.0	2.2	2.9	3.1	.80	.83	.90	.95	7.0	7.1	7.4	7.0	4
9–11%	2.8	2.6	2.6	2.6	.66	.70	.78	.82	6.4	6.5	6.6	6.4	5
13–20%	2.8	2.7	2.6	2.4	.60	.64	.72	.70	6.4	6.4	6.5	6.5	5
22–35%	2.8	2.6	2.7	2.4	.52	.66	.72	.72	6.3	6.4	6.7	6.7	5

a tendency to increase with the application of phosphorus. Since the RSA/yield ratio is decreasing and $\bar{C}$ for nitrogen is constant (Table 1) it seems clear that increased phosphorus supply to the plant resulted in a marked increase in the plant demand coefficient for nitrogen.

The tendency for % K to be higher in general in the low response group of sites may be the result of a higher potassium status of the soil at these sites. A similar explanation may apply to % N but in the case of % N the tendency can also be explained by the increase in the plant demand coefficient for nitrogen associated with the higher phosphorus status of these sites.

4.3 *Discussion*

The foregoing interpretation of the results indicates that percentage nutrient content in the plant is not uniquely related to the level of supply of the nutrient in the soil. The following assumptions have been made here:

(a) that the final root surface area was an estimate of the root surface that was active in uptake during the season;
(b) that the weight of fibrous roots could be estimated from a simple relation with the weight of the swollen part of the swede root;
(c) that the weight of the fibrous root system provided an estimate of surface area;
(d) that the nutrient content of the leaves provided a measure of the nutrient content of the whole plant;
(e) that application of each fertiliser had no effect on soil availability of other nutrients.

From the considerations presented in the introduction and from our experience we feel that these assumptions are at least approximately true. Because these assumptions, which require verification, have been made, the equations given in Table 1 have been used only in a qualitative way. A semi-quantitative assessment of these effects in the phosphorus experiment may be obtained from Figures 2 and 3 where % P has been plotted against the

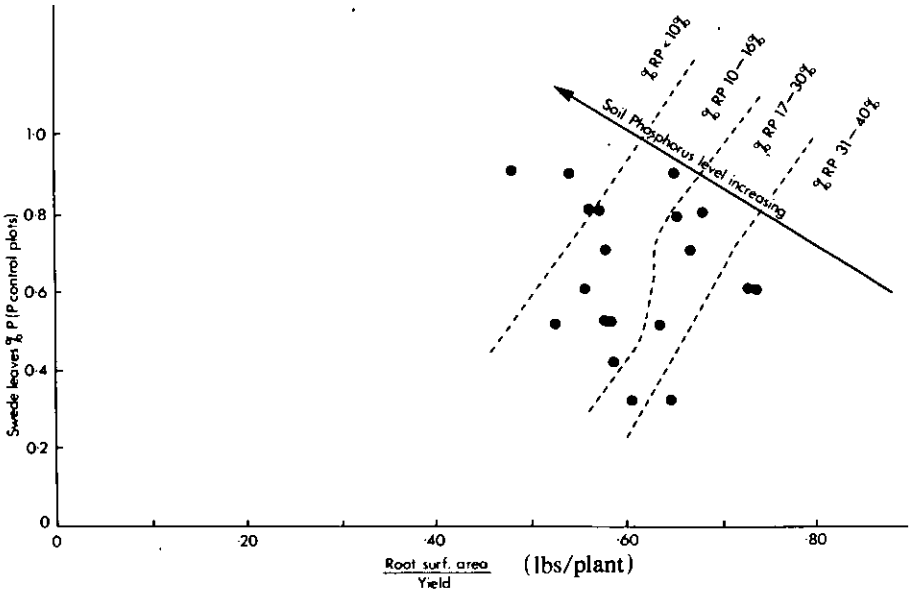

Figure 2. Relation between % P in the plant and RSA/yield ratio at several levels of soil phosphorus (soil phosphorus level indicated by % response to P).

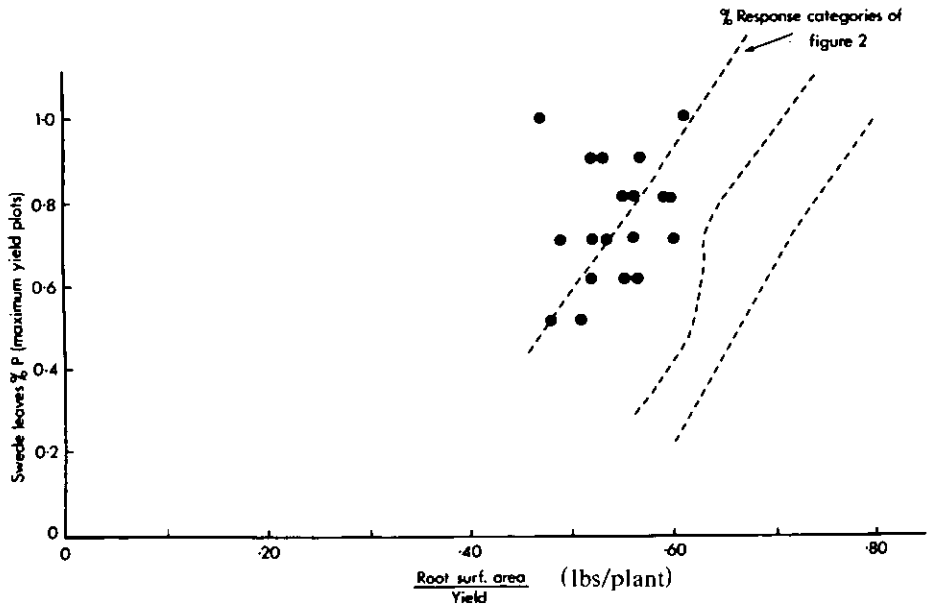

Figure 3. Relation between % P in the plant and the RSA/yield ratio when yield restriction by phosphorus is removed (i.e. at maximum yield with phosphorus applied).

root/yield ratio. Approximately, the slope of the line relating % P to the ratio should provide a measure of $a_p\bar{c}_p$. Figure 2 refers to the control plots so that $\bar{c}_p$ was varying relatively widely between sites. In Figure 3, which uses data only from the maximum yield plots, $\bar{c}_p$ was constant between sites. The period t during which any portion of root surface is active in uptake is always constant.

It is notable that in Figure 2 the variation in % P induced by variation in the RSA/yield ratio in each response category covered almost the same range of values as variation in % P between response categories. In Figure 3, where soil phosphorus level at each site was brought to the level sufficient to give maximum yield the % P variation covered much the same range of values.

The effect of phosphorus on the ratio of RSA/yield is in keeping with the suggestion [2] that increased nutrient supply to the plant generally has this effect. The poor correlation between % P and soil P (Figure 1) may be taken to be the result of the compensating effects of the reduction in the RSA/yield ratio and plant demand coefficient as the phosphorus supply increased and of variations in the ratio in response to uncontrolled soil and climatic variations between sites.

The absence of any effect of potassium on the ratio possibly reflects the role of potassium in the plant as a factor in leaf photosynthesis. The decrease in the ratio in response to increases in nutrient supply has been interpreted as a mechanism for the maintenance of a carbohydrate-mineral balance in the plant. In the case of potassium – its effect in increasing photosynthetic efficiency of the leaf renders an adjustment of the ratio unnecessary. The tendency for potassium to accumulate to very high level (luxury consumption) may be taken to be a reflection of its failure to reduce the ratio of RSA/yield and this may also explain the fact that % K gives a better correlation with soil K than in the case of phosphorus (Figure 1).

Bibliography

1. *Nye, P.H.*, and *Tinker, P.B.*: The concept of a root demand coefficient. J. Appl. Ecol. 293–300 (1969).
2. *Troughton, A.*: Measurement of plant response to mineral nutrition. International Society of Soil Science, meeting of commissions II and IV, Aberdeen 1966 (Ed. G.J. Jacks), pp. 385–91, Aberdeen, 1967.
3. *Brouwer, R.*: Root growth of grasses and cereals. In: The growth of cereals and grasses, Proceedings of the 12th Easter School in Agricultural Science, University of Nottingham (Ed. F.L. Milthorp & J.D. Ivins), pp. 156–66, London, 1966.
4. *Bleasedale, J.K.A.*: Plant growth and crop yield. Ann. appl. Biol. *57*, 173–82 (1966).

The Distribution of Potassium, Calcium, and Magnesium between Aerial Organs of Five Grasses at Early Maturity

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1. *Introduction*

Potassium is unique in being the only univalent cation that is indispensable for the growth of all living organisms. Its content in plant leaves varies over a wide range and may reach levels of 10 per cent and over in such species as tomatoes and sugar beet. Such levels have been recorded at this station for commercially grown crops [*11*]. It is not surprising therefore that in general, its level in plants exceeds that of any other cation. There is abundant evidence that potassium functions as a cofactor for a variety of enzymes involved in such metabolic processes as carbohydrate, nucleic acid, and protein synthesis, and while this may account in part for its generally high levels in plants, it is also true that potassium is one element that is frequently absorbed in luxury quantities. In the plant it occurs mainly as soluble inorganic salts and to a lesser extent as salts of organic acids. Its role in plant physiology has been reviewed in a submission to a previous *International Potash Institute Conference* by *Fowler* [*6*] while more recent reviews include those of *Nason* and *McElroy* [*10*] and *Evans* and *Sorger* [*3*].

Calcium and magnesium are related to potassium in plant nutrition and it seems expedient to consider the three elements together.

Calcium also acts as a cofactor in enzyme systems but is also regarded as having a structural function in the plant where, as calcium pectate, it occurs as a cell wall constituent. Magnesium is probably best known as a constituent of chlorophyll but it is also required non specifically by a large number of enzymes involved in phosphate transfer.

Work on the distribution of the above mentioned elements in other grass species has been reported by the author [*4*] while more recent studies involving distribution at different growth stages have been recorded by *Davey* and *Mitchell* [*2*] and by *Pritchard, Pigden* and *Folkins* [*13*].

The present paper deals with distribution in grasses not reported in the previous study [*4*] and endeavours to lay emphasis on the contribution of the different aerial organs to whole plant contents. The significance of mineral distribution from the animal nutrition viewpoint is also discussed.

2. *Experimental*

The five grasses studied were Bent grass *(Agrostis tenuis)* Yorkshire fog *(Holcus lanatus)* Crested dogstail *(Cynosurus cristatus)* Italian ryegrass *(Lolium Italicum)* and Rough

stalked meadow grass *(Poa trivialis)*. The species were grown singly in plots (5 × 5 m) and representative samples taken at early maturity i.e. when heads had formed but before any seeds had shed. Basal fertilizer was applied as follows; 2 kg/ha calcium ammonium nitrate, 5 kg/ha superphosphate and 2 kg/ha potassium chloride. The experiment was in the form of a randomized block on an acid brown earth of medium base status (Dystrocrept). The pH over the site varied between 6.0 and 6.2. Samples were separated by hand into heads, leaves (+ sheaths) and stems, dried at 80 °C and ground in a stainless steel mill. Potassium and calcium were determined by flame photometer while magnesium was determined colorimetrically using thiazol yellow. All results are reported as percent on oven dry matter.

3. *Results and Discussion*

The actual contents of K, Ca and Mg are shown in Table 1 and the variation (t-test) in contents between the different organs, in Table 2.

It will be apparent for Ca that no statistical variation could be recorded between any organs in Yorkshire fog and between head and stem in Italian ryegrass. This situation arose because the replicate analyses were in fact identical but an inspection of the actual contents shown in Table 1 reveals that the differences between the organs are relatively large. For instance the Ca content of the leaf of Yorkshire fog is twice that of the head which in turn is twice that of the stem. Differences of roughly similar magnitude are apparent in the case of Italian ryegrass. Table 3 shows the percentage contribution of the different organs to total dry matter and to total K, Ca and Mg.

Table 1. Potassium, calcium and magnesium in different organs of five grasses*

	(% on dry matter)								
	K			*Ca*			*Mg*		
Species	Heads	Leaves	Stems	Heads	Leaves	Stems	Heads	Leaves	Stems
Agrostis	1.3	1.4	1.5	0.33	0.80	0.13	0.17	0.16	0.11
Yorkshire fog	1.4	2.1	2.2	0.40	0.80	0.20	0.22	0.22	0.12
Crested dogstail	1.9	2.3	2.5	0.17	0.93	0.27	0.16	0.22	0.13
Italian ryegrass	1.1	1.9	1.1	0.50	1.20	0.30	0.14	0.18	0.08
R.S. meadow grass	1.7	2.5	2.1	0.47	1.13	0.30	0.18	0.26	0.10

* Averages of three replications

Table 2. Variation of potassium, calcium and magnesium between different plant organs

	K			*Ca*			*Mg*		
Species	H-L	H-S	L-S	H-L	H-S	L-S	H-L	H-S	L-S
Agrostis	—	—	—	**	—	**	—	**	**
Yorkshire fog	**	*	—	Z.V.	Z.V.	Z.V.	—	**	*
Crested dogstail	*	**	—	**	—	**	*	—	—
Italian ryegrass	**	—	*	**	Z.V.	**	*	**	**
R.S. meadow grass	**	*	*	**	*	**	*	**	**

** P = .01 * P = .05 Z.V. = zero variance between replications — = not significant

Table 3. Percentage contribution of heads, leaves and stems to total dry matter and to total content of potassium, calcium and magnesium.

Species	Dry matter			K			Ca			Mg		
	H	L	S	H	L	S	H	L	S	H	L	S
Agrostis	17	14	69	16	13	71	22	42	36	33	19	59
Yorkshire fog	19	16	65	13	18	69	19	41	40	42	23	51
Crested dogstail	16	18	66	12	18	70	7	42	51	30	26	57
Italian ryegrass	14	13	73	10	16	74	16	36	48	34	24	56
R.S. meadow grass	24	15	61	20	19	61	24	36	40	48	28	42

H = Heads L = Leaves S = Stems

The statistical data in Table 2 reveal some clearcut differences between the five grasses. In the case of Agrostis no significant differences in potassium occurred between any of the organs while with rough stalked meadow grass differences were apparent between all organs. Viewing the grasses as a group it may be said that the differences between organs were less marked for potassium than for either calcium or magnesium (four differences at the 1 per cent level for K as against eight for Ca and seven for Mg). This is held to be a direct reflection of the greater mobility of potassium within the plant compared with either calcium or magnesium. A similar analysis carried out for the different elements when present in both limiting and luxury supply might however prove rewarding.

When the contribution of the different organs to the total dry weight and to the total contents of K, Ca and Mg, is studied, some interesting differences emerge. In the case of both dry matter and potassium the stems of all species contributed some 70 per cent of totals. The remaining 30 per cent was shared between heads and leaves in proportions varying approximately between equality and 1.5 to 1.

With calcium, leaves and stems contributed about 80 per cent of the total, and in the case of *Agrostis,* Yorkshire fog and rough stalked meadow grass, this amount was shared relatively evenly between leaf and stem.

The magnesium data reveal that stems contributed over half the total and leaves quite clearly the least. This relatively low contribution of the leaves to the total magnesium content might at first sight appear strange but with increasing plant maturity magnesium is known to move from vegetative to reproductive tissue where it is used in seed formation. At this stage of maturity the leaf and head contributions of phosphorus are very similar to those of magnesium (5) and in this respect it is interesting to recall that magnesium is required for enzymatic reactions involving group transfer, namely those in which phosphate participates [*10*].

Further studies on the mode of combination of nutritionally important elements in different plant organs certainly seem warranted. Apart from increasing our knowledge of general plant chemistry there is the practical question of how elements in different forms of combination are available to the grazing animal. That elements in different plant parts, and in plants at different stages of maturity, can vary in digestibility has been shown by several workers [*9, 12, 14, 15*]. Using *in vitro digestibility (I.V.D.)* techniques, *Mowat, Christie* and *Winch* [*9*] showed that live leaves had a higher I.V.D. than dead leaves which in turn had a higher I.V.D. than heads or stems. *Terry* and *Tilley* [*14*] pointed out that with advancing maturity, the digestibility of stems falls faster than that of heads and a decline in digestibility was associated with a reduction in the content of water soluble and protein constituents in the plant. A biochemical basis for selection of forage of high digestibility is inherent in this work. While the bulk of digestibility studies have been

concerned with the organic components of plants, the availability of mineral elements has received attention from such workers as *Hutton, Jury* and *Davies* in New Zealand [*7, 8*] and *Armstrong, Thomas* and *Armstrong* in England [*1*]. *Hutton et al.* showed that with lactating cows, the retention of magnesium was as low as 8 per cent while that of potassium was 50 per cent and calcium 65 per cent while Armstrong et al. pointed to the differing availability of calcium in three common grasses.

It is clear that the studies briefly outlined above form the basis for a relatively unexplored field of work which would endeavour to relate the form of combination of nutrient elements in plants and their component parts to their assimilation by the animal. The primary aim of this paper is to focus attention on this area of research.

Bibliography

1. *Armstrong, R.H., Thomas Brynmor* and *Armstrong, D.G.:* The availability of calcium in three grasses. J. agric. Sci. Camb. *49*, 446–453 (1957).
2. *Davey, B.G.,* and *Mitchell, R.L.:* The distribution of trace elements in cocksfoot *(Dactylis glomerata)* at flowering. J. Sci. Fd. Agric. *19*, 425–431. (1968).
3. *Evans, H.J.,* and *Sorger, G.J.:* Role of mineral elements with emphasis on the univalent cations. A. Rev. Pl. Physiol. *17*, 47–76 (1966).
4. *Fleming, G.A.:* Distribution of major and trace elements in some common pasture species. J. Sc. Fd. Agric. *14*, 203–208 (1963).
5. *Fleming, G.A.:* Uptake of nutrients by some pasture species. Res. Rept. Soils Divn. Agric. Inst. (Ireland), 60 (1963).
6. *Fowler, H.D.:* The role of potassium in plant nutrition. Proc. 1st. Colloquium of the Int. Potash Inst. Wexford (Ireland) 25–33 (1963).
7. *Hutton, J.B., Jury, K.E.,* and *Davies, E.B.:* Studies of the nutritive value of New Zealand dairy pastures 4. The intake and utilization of magnesium in pasture herbage by lactating dairy cattle. N.Z. Jl. agric. Res. *8*, 479–496 (1965).
8. *Hutton, J.B., Jury, K.E.,* and *Davies E.B.:* Studies of the nutritive value of New Zealand dairy pastures 5. The intake and utilization of potassium, sodium, calcium, phosphorus and nitrogen in pasture herbage by lactating dairy cattle. N.Z. Jl. agric. Res. *10*, 367–388 (1967).
9. *Mowat, D.N., Christie, B.R.,* and *Winch, J.E.:* The *in vitro* digestibility of plant parts of orchardgrass clones with advancing stages of maturity. Can. J. Pl. Sci. *45*, 503–507 (1965).
10. *Nason, A.,* and *McElroy, W.D.:* Modes of action of the essential mineral elements. In 'Plant Physiology'. Ed. F.C. Steward, Vol. 3, pp. 81, Academic Press, 1963.
11. *Parlè, J.:* Private communication.
12. *Pritchard, G.I., Folkins, L.P.,* and *Pidgen, W.J.:* The *in vitro* digestibility of whole grasses and their parts at progressive stages of maturity. Can. J. Pl. Sci. *43*, 79–87 (1963).
13. *Pritchard, G.I., Pigden, W.J.,* and *Folkins, L.P.:* Distribution of potassium, calcium, magnesium and sodium in grasses at progressive stages of maturity. Ibid. *44*, 318–324 (1964).
14. *Terry, R.A.,* and *Tilley, J.M.A.:* The digestibility of the leaves and stems of perennial ryegrass, cocksfoot, timothy, tall fescue, lucerne and sainfoin, as measured by an *in vitro* procedure. J. Brit. Grassland Soc. *19*, 363–372 (1964).
15. *Wilson, R.K.,* and *McCarrick, R.B.:* A nutritional study of grass swards at progressive stages of maturity 1. The digestibility, intake, yield and chemical composition of dried grass harvested from swards of Irish Perennial ryegrass, timothy and a mixed sward at nine progressive stages of growth. Ir. J. agric. Res. *6*, 267–279 (1967).

Discussion, Session No. 2

Dr. *R. Scott Russell* (Wantage/England):
Prof. *Mengel* has asked me to comment further on the hypothesis that salts are absorbed in plants by two carrier mechanisms, one being operative at low concentrations the other at high concentrations. These views of course rest primarily on relationships observed between the external concentration and ion uptake in detached root systems or tissue slices. There seems no doubt that a 'break' in the curve occurs at intermediate concentrations (usually above 0.5–1.0 meq/l). However, the interpretation of the two hyperbolic curves for the lower and upper concentration ranges seems less certain. Work on this subject has normally been done under non-sterile conditions and in my paper with Dr. *Clarkson* we compared the relationships which *Barber* obtained under sterile and non-sterile conditions for the lower concentration range; both the uptake by sterile roots and the additional uptake of micro-organisms on non-sterile roots are of the general form which, on the basis of the *Michaelis-Menten* analysis, has been assumed to imply a single mechanism. However, the large contribution of micro-organisms at the lower concentrations indicates that work must be carried out under rigidly sterile conditions if precise conclusions on plant function are to be reached.
The concentrations at which the second mechanism dominates are above those which can, in general, be regarded as in the physiological range. I entirely agree with Professor *Mengel* that this mechanism may well be due solely to the passive diffusion of ion in the tissues under these abnormal circumstances. It is perhaps surprising that this possibility was not examined in detail before the two mechanisms were proposed and it is of interest, as noted in our paper, that *Higinbotham* and his colleagues found evidence of the active transport of potassium only in the lower concentration range.

Dr. *H.E. Haeder* (Hannover/Federal Republic of Germany):

In your last figure we saw a decrease of the potassium content in the plants with time. You pointed out that this must be lost by leaching out of the leaves and by efflux of the roots. In our former experiments with maturing barley plants in hydroponics we found only a small efflux from the roots (less than 1%). Therefore the decrease of potassium content with time will be due only to the leaching of the leaves.

Mr. *F. van Egmond* (Wageningen/The Netherlands)
(replying to the question of Dr. *Kirkby*):

When sugarbeet plants are grown in nutrient solution with NO_3^- as nitrogen source, the pH of the solution will hardly change, (C.A.) and organic N will have the same value for the whole plant. Conclusion: cations and anions are taken up in equal amounts.

Prof. Dr. *H. Marschner* (Berlin/Germany):

In your experiments most of the ^{14}C-assimilates which moved to the fruit were sugars, the amount of ^{14}C-organic acids was low. Do you think that despite of this, there were still enough organic acid anions moving in the phloem as counter ions to account for the simultaneous high flow of K^+ in the phloem to the growing fruit?

Dr. *H.E. Haeder* (Hannover/Federal Republic of Germany):

There are two hypothesis for the reaction of K^+ on translocation of carbohydrates in plants.
1) *Kursanov* and co-workers found an enzyme in the phloem sap (Fructokinase) which catalyses the translocation of fructose through the cells to the veins. This enzyme is stimulated by K^+.
2) The other opinion deals with the 'turnover' of the transported sucrose from the veins to the storage tissues. Our recent results confirmed this hypothesis. We observed a faster turnover of the labelled ^{14}C from sucrose to other compounds in the tomatoes.

Dr. *L.K. Wiersum* (Haren-Groningen/The Netherlands):

Evidence for Na-extrusion might be related to the fact that Na-application or Na-arrival in a leaf of a glycophyte is followed by partial efflux out of the leaf through the phloem.
The retaining Na could be extruded into the vacuole as it seems to be not readily exchangeable.

Dr. *L.K. Wiersum* (Haren-Groningen/The Netherlands)
in reference to equivalency of K and sugars in phloem:

Data obtained in the group of *Tammes* and *van Die* on yucca phloem sap demonstrate that the amount of sugars (10—15%) is far higher than K. The K content seems indeed to be balanced by the mixture of organic and inorganic ions. Complete data will be published as they are being investigated at this moment.

Dr. *A. Reinberg* (Paris/France):

Biological rhythms have been demonstrated objectively in plants as well as in animals including men. For any metabolic study in men, and particularly for that of potassium we have to take into consideration the existence of periodic and thus predictable variations. Therefore my question to plant physiologists is: as methodologic background, is it important or not, when studying (among others) potassium metabolism to consider the clock hour of the experiment in relation to regime of light — dark alternation?

Prof. Dr. *W. Höfner* (Gießen/Federal Republic of Germany):

To answer the question of Mr. *Reinberg*, concerning recognition of cyclic processes in plant physiology studies, I want to draw your attention to the exudation experiments done in our department. According to the results of *Herwig*, the well known diurnal fluctuation of exudation rates of decapitated sunflower plants is paralleled with the amount of K within this exudate. K is the only element, which concentration goes along with the exudation rate, and this points to the osmoregulation function of this element.

Co-ordination Lecture for Session No. 2

Prof. Dr. K. MENGEL, Head of the Agricultural Research Station Büntehof, Hannover/Federal Republic of Germany.

The papers presented at the 2nd session obviously demonstrate that there was a real progress during the last decade in understanding ionic relationships in the plant. As was shown by *Coïc's* paper the nitrogen nutrition and the nitrogen metabolism controls the uptake and distribution of inorganic ions to a high extent. Even the plant organ in which the nitrate is reduced predominantly plays a role in cation uptake and cation translocation from roots to the upper plant parts. It further appears that a balance between cations and anions is a prerequisite for normal plant life. This balance relates to the gross uptake and release of cations and anions by plant roots as to the balance between cations and anions in various plant parts as well. The ionic balance at the uptake of ions from the nutrient solution is mainly a balance between inorganic ions, whereas in the various plant parts the organic anions represent a major anion fraction in balancing the bulk of cations. This is especially true when nitrate is the main N source of the plant. The anion equivalent of the nitrate shifts over to the anion equivalent of an organic anion during nitrate-N assimilation. This relationship became evident in *Van Egmond's* data, showing that the amount of organic anions was nearly identical the amount of organic N. This correspondence has to be expected, provided the release of OH^- or HCO_3^- or the uptake of H^+ by the roots is negligible.

A further important factor in ionic relationships is the ability of ions to be transported in basipetal direction. *Van Egmond's* graphics showed that older leaves have higher contents of oxalate that younger ones, which he explained by the low solubility of the oxalates. It remains open, whether this explanation is sufficient, as plant species having malate as the main organic anion also show a higher organic anion content in older leaves.

Ion content and ion distribution cannot only be explained by the N metabolism, but depend largely on the ability to be actively transported through biological membranes. As was outlined clearly by the main speaker *Scott Russell* potassium can be transported against an electrochemical potential gradient, whereas for calcium and sodium such an active uptake by cells of higher plants has not yet been proved. This unique feature of potassium absorption enables the root to take up potassium with high rates even from rather dilute solutions. This property is inherent in plant roots along the whole range from the apical to the basal part, and a short root segment of this range can supply the entire plant with potassium, provided the potassium supply from the environment to this root segment is sufficient. Therefore rather the potassium transported to the roots than the root surface controls the total potassium uptake. In agreement with this deduction *Scott Russel's* data showed that the relationship between the potassium uptake and the root surface or the root length is poor. The best relationship exists between the root volume and the potassium uptake. This is surprising and the question which parts of the plant roots

and to what extension under field conditions are related to the total ion uptake needs further investigations.

The unique feature of potassium to be accumulated in plant cells by active uptake mechanisms affects the water economy too. As was reported by *Höfner* an accumulation of potassium in the xylem vessels effects an osmotic gradient, which is responsable for the water uptake from the root environment and for the root pressure. Also the potassium accumulation in the guard cells of the stomata, effecting stomata opening and closing plays a major role for the water economy of the plant. The film and the comments of *Trolldenier* illustrated the stomata movement obviously.

The high potassium concentration found in sieve tube vessels justifies the assumption that potassium is secreted into the sieve tube vessels actively. Here it is the dominant cation balancing the various organic anions. It is possible that the favorable influence of potassium on the transport of photosynthates from the leaves to the fruits, clearly shown by Miss *Viro's* data, is caused by this balancing effect. On the other hand it still remains clarified, whether the potassium for the transport of organic compounds in the phloem tissue has still a more specific function.

3rd Working Session	**Potassium in Biochemistry and Physiology of Animals**
Chairman of the Session:	Prof. Dr. *R. Bach*, Institute of Agricultural Chemistry, Federal Institute of Technology, Zurich/ Switzerland; Member of the Scientific Board of the International Potash Institute.

Ion Transport through Biological Membranes

Dr. VALBORG KOEFOED-JOHNSEN, lecturer, and Prof. Dr. H.H. USSING, Institute of Biological Chemistry A, University of Copenhagen/Denmark

Summary

The properties of some ion transporting epithelia are discussed. The isolated frog skin and the urinary bladder of toad are extensively used as examples, since much information about active transport processes have been obtained from studies of these tissues.
The electric potential difference between the outside and inside of the isolated frog skin bathed with Ringer solution on both sides is maintained by active transport of sodium from the outside to the inside solution.
The varying hypotheses for the development of the potential difference are discussed. It is concluded that the frog skin seems to possess a variable intercellular shunt path which together with the cellular shunt and the active sodium transport mechanism determines the level of the frog skin potential.

It is a well-known fact that it is of vital importance for all cells to maintain a constant ionic composition. Most cells, both plant and animal cells, possess the ability to accumulate potassium and exclude sodium, and they are able to maintain the constancy of their internal ionic environment even though their cellular composition of inorganic ions differs greatly from that in the surrounding medium.

During the last half century much interest has been focused on the problem of ion accumulation and secretion. The advanced analytical and electronic techniques of the recent years have added greatly to our knowledge of transport processes and their regulation, and the literature dealing with the subject is immense. (For reviews covering the field of transport phenomena see a.o the following: *Ussing et al.* [*37*], *Comar* and *Bronner* [*7a*], and *Keynes*[*15*].)

For the present we shall mainly discuss the properties of some epithelia which have in common the capacity that they can transport sodium from their physiological outside to their inside. The tissue comprises such a variety as e.g. the skin and urinary bladder of amphibians, intestinal mucosa, gallbladder, and the epithelium of rumen in ruminants. Since much information about active ion transport has been obtained from studies on toad bladder and frog skin the following discussion of basic transport properties will especially deal with observations originating from these tissues.

The isolated frog skin has during the last twenty years or more been the object for transport studies in our laboratory, and has proved to be a valuable preparation for examining general features of transport processes through epithelia. By means of this preparation it has been proved that active transport of sodium is the source ot the potential across the frog skin *(Ussing* [*30*]*)*, and by use of the short-circuit technique *(Ussing* and *Zerahn* [*34*]*)* it was shown that the net flux of sodium ions can account for the current

drawn from the skin, i.e. that sodium is the main ion actively transported. Flux ratio analysis *(Ussing [31])* of chloride ion movements has shown that these ions move passively through the isolated skin *(Koefoed-Johnsen, Levi,* and *Ussing [19])*. The active transport seems to be energized mainly by oxidative metabolism, and the stoichiometry between sodium transported and oxygen consumed has been established to be 18 sodium ions per 1 molecule of oxygen *(Zerahn [41])*.

1. *The nature of the frog skin potential*

On basis of knowledge of the overall picture of ion transport through epithelial cell layers and of single cell systems the so called two membrane hypothesis was put forward in order to explain the origin of the frog skin potential *(Koefoed-Johnsen* and *Ussing [21])*. The spontaneous potential across the isolated frog skin bathed on both sides with *Ringer* solution is considered to be the result of active transport of sodium ions and the shunting effect of passively moving ions like chloride ions. The maximal obtainable potential will consequently develop only in the ideal case where there is no shunting by passively diffusing ions.

The hypothesis is based on the following assumptions:

1) A continuous cell layer forms the barrier between the inside and outside solution.
2) The outward and inward facing membranes of these cells have different selectivities.
3) The outward facing membrane is selectively but passively permeable to sodium, and virtually impermeable to all other cations (except lithium), and furthermore rather permeable to chloride and other small anions.
4) The inward facing membrane is highly permeable to potassium and small anions while the passive permeability to sodium is very low.
5) The active transport is located at the inward facing membrane and is visualized as a sodium-potassium exchange pump (Figure 1).

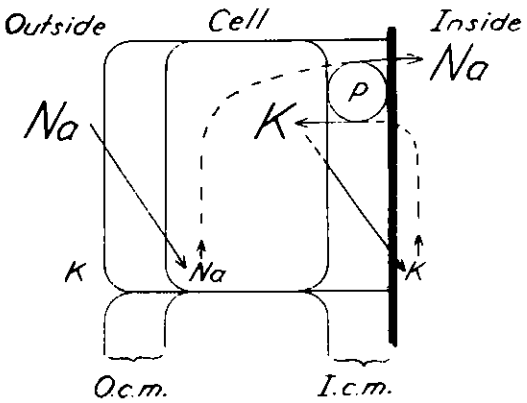

Figure 1. Diagram of an epithelial cell. The concentration levels of the cations are indicated by the size and the level of the chemical symbols. The oblique arrows designate passive, but highly specific diffusion. P is a 'pump' mechanism.

From these assumptions it follows that the electrical potential which the sodium pump develops across the epithelium in absence of penetrating anions should be composed of two potentials: One between the outside medium and cell interior determined by the respective sodium concentrations, and another between the cell interior and the inside solution dependent on the respective potassium concentrations. The potential across the skin would then be the sum:

$$E = RT/F\left(\ln\frac{(Na)_o}{(Na)_c} + \ln\frac{(K)_c}{(K)_i}\right),$$

subscripts o, c, i denoting outside, cell, and inside.

The experimental evidence on which the hypothesis is based is o.a. the following. The selectivities of the outward and inward facing membranes can be demonstrated when the shunting effect of the anions is stopped, either by treating the outside of the skin with copper at a concentration of 10^{-5} M which decreases the chloride permeability considerably *(Koefoed-Johnsen* and *Ussing* [*21*]*)*, or by substituting a virtually non-penetrating anion like sulfate for chloride. Under these conditions a frog skin from *R. temporaria* can obtain very high potentials (occasionally as much as 180 mV), and responds to changes in the ionic composition of the bathing media as if the outside surface were a sodium electrode and the inside a potassium electrode. In agreement with the model the resistance of the skin increases considerably when sodium in the outside solution is replaced by potassium *(Ussing* and *Windhager* [*36*]*)*. Volume changes of the frog skin epithelium produced by altering the K/Na ratio in the presence of penetrating and non-penetrating anions can also be satisfactorily explained in accordance with the model *(MacRobbie* and *Ussing* [*25*]*)*.

The effects of many inhibitors and stimulators of the sodium pump can also be explained in terms of the model. The potential *(Fukuda* [*12*]*)* and the sodium transport (Koefoed-Johnsen [*54*]*)* are dependent on the presence of potassium in the inside bathing solution and are highly reduced in the absence of potassium. The cardiac glycoside ouabain which is generally accepted as a specific inhibitor of Na/K pumps inhibits the sodium transport in frog skin and abolishes the potential when added to the inside solution, but has no effect when added to the outside *(Koefoed-Johnsen* [*17*]*)*. The diuretic amiloride is a strong inhibitor of sodium transport. Its effect can only be seen when the drug is added to the mucosal side of the toad bladder *(Bentley* [*3*]*)* or to the outside of the frog skin *(Baba et al.* [*1*]*)*, *Nielsen* and *Tomlinson* [*27*]*)*. The action of amiloride is attributed to a restriction of the entry path for sodium. Addition of neurohypophyseal hormones to the inside bathing solution of the frog skin leads to an increased active transport of sodium *(Ussing* and *Zerahn* [*34*]*)* and an increased permeability to water *(Koefoed-Johnsen* and *Ussing* [*20*]*)*, and the same effects are found in toad bladder *(Leaf* and *Dempsey* [*23*], *Bentley* [*2*]*)*. The stimulating effect of these hormones has also been explained in terms of the model *(MacRobbie* and *Ussing* [*25*], *Frazier, Dempsey* and *Leaf* [*11*]*)*: The hormones increase the permeability of the outer border leading to an increased cellular concentration of sodium, which in turn stimulates the pump to an increased activity.

That a permeability change is involved in the reaction has been demonstrated in toad bladder *(Civan* and *Frazier* [*7*]*)* where the fall in electric resistance was found to occur at the mucosal surface. Under conditions of zero net sodium transport obtained by reducing the electrochemical gradients for chloride across the tissue to zero, vasopressin was found to decrease the electrical resistance as expected, but not to increase the transepithelial

potential. This indicates that the effect of the hormone is not a direct stimulation of the pump *(Civan, Kedem,* and *Leaf* [6]). Recently the effect of vasopressin has been reinvestigated on isolated sheets of epithelial cells and isolated epithelial cells scraped from toad bladder *(MacKnight, Leaf,* and *Civan* [24]). After exposure to vasopressin these cells contain significantly more sodium, chloride, and water; and the concentrations of the ions are also increased. The cellular potassium is not affected, though its concentration fell significantly. These findings would be expected if vasopressin increases the permeability of the cells to sodium, whereas the cells would have lost sodium and would have been shrunk, if the hormone had stimulated the extrusion of sodium by the pump.

2. *Localization of the ion selective membranes in the frog skin*

Although many observations have been satisfactorily explained in light of the hypothesis, others do not fit the pattern so well.

Originally the outer and inner ion selective membranes were thought to be the outer and inner cell membranes of the *stratum germinativum,* the basal layer of the frog skin. The assumption was in agreement with intracellular potential measurements made by means of *Ling Gerard* type electrodes *(Engbæk* and *Hoshiko* [9]). In most cases only one stable plateau was found within the skin epithelium. However, in later micropuncture studies *(Ussing* and *Windhager* [35]) several stable plateaus were found on the advancement of the electrode from the outside, the first potentials encountered being consistently more negative than the following ones.

Potassium exchange studies furthermore seem to indicate that the sodium selective, potassium impermeable membrane must be situated further towards the outside of the skin, right underneath the very thin cornified cell layer *(Koefoed-Johnsen* [18]). In these experiments the inside of the frog skins was exposed to *Ringer* containing ^{42}K. After varying times of exposure the skins were rapidly frozen onto a horizontal area of solid agar on the stage of a freezing microtome and cut into thin sections parallel to the outer surface of the skin. The specific activity of ^{42}K in the single sections was then measured. It turned out that the specific activity of potassium was the same throughout the epithelium whether the skin had been exposed to ^{42}K for 15 minutes or 3 hours (Table 1). In other words: All epithelial cell layers could exchange their potassium with that of the inside bathing solution. In cases where the isotope had been added to the outside bathing solution there was no measureable uptake by the skin after the same periods of time.

Table 1. Abdominal skins from six R. temporaria activated with ^{42}K in Ringer solution from the inside*

Skin No.	Activation time (min)	Per cent exchange of K between epithelial layers and inner solution		
		Section 1, basal layer	Section 2, middle layer	Section 3, outer layer
1	15	22	23	27
2	15	30.3	30.3	29.5
3	180	81.7	87	—
4	180	92.2	99	—
5	180	98.3	100	—
6	180	101	96	—

* After varying periods of time the skins were cut on a freezing microtome into sections (20 μ) parallel to the skin surface *(V. Koefoed-Johnsen [18]).*

On the basis of these and other observations the following revision of the model was proposed *(Ussing* and *Windhager [36]):* The epithelial cells are interconnected by intercellular bridges of low resistance, while the intercellular space forms a continuous system of channels which communicate rather freely with the inside bathing solution through pores in the basement membrane. Towards the outside the interspace system is more or less completely closed by *zonulae occludentes,* the 'tight seals', between the cells of the first living cell layer. This model is in many respects in agreement with that proposed on basis of electron-microscopical studies *(Farquhar* and *Palade [10]).*

In the revised model (Figure 2) the sodium selective membrane has been moved to the outside facing membrane of the first living cell layer, while the potassium selective membrane with the sodium pump would be the total surface of all epithelial cells facing the intercellular space. The picture is in agreement with the finding that all ATPase can be found located along the surface of the cells lining the interspace, but not along the outer surface of *stratum corneum* or along the inner surface of *stratum germinativum (Farquhar* and *Palade [10]).*

The model is, however, still not quite satisfactory. Thus is has been pointed out that the transport pool of sodium in the epithelium seems to be much smaller than the total epithelial sodium content *(Cereijido* and *Rotunno [5], Zerahn [42]).* The discrepancy might be explained in terms of compartmentalization of some part of the sodium. Thus it is known that nuclei concentrate sodium preferentially *(Mirsky* and *Allfrey [26]).* Another

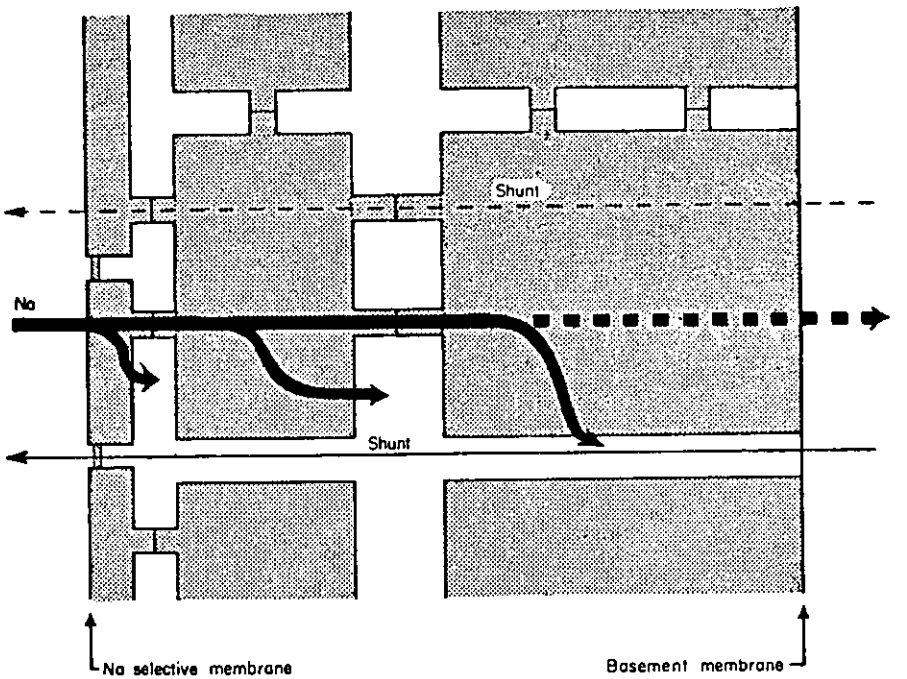

Figure 2. Diagram of the frog skin epithelium, showing the active transport path, the cellular and the intercellular shunt spaths.

explanation could be that the outermost living cell layer performs the greatest part of the sodium transport.

The question whether all cell layers participate to the same extent in the transport of sodium can still not be answered with certainty. Some experiments with bearing on this problem have been made, however *(Voûte* and *Ussing [38–39])*. If the sodium transport is increased by passing an inwards going current, e.g. double the short circuit current through the skin, the cells in the first living cell layer swell, while the rest of the epithelium is increased by passing an inward going current, e.g. double the short circuit current shrink with no significant change in deeper cell layers. These results seem to suggest that the main part of the sodium transport takes place in the reacting cells. It remains though to be proven that the changes are due to changes in sodium content.

3. *The interspace system*

Whether only one or several cell layers participate in the transport process all transported sodium has, according to the model, to leave the epithelium via the interspaces. Accordingly the shape and volume of the interspace system might influence the transport rate, or reversely, the transport rate might influence the shape of the interspace.

Some experiments elucidating this relationship have been made *(Voûte* and *Ussing [40])*. Small hydrostatic pressures (of 5–40 cm) of water were applied to the inside of frog skins leading to a reversible expansion of the interspace the volume of which increases with increasing pressure. In spite of the drastic changes in interspace volume the short circuit current and thus the active transport remains constant. The potential is unaffected in some skins, in others the stretching of the skin leads to a reversible decrease in potential, possibly caused by the creation of a shunt through the *zonulae occludentes*. Such a leak would not measurably affect the current output during short circuiting, but would diminish the potential during open circuit.

The question of the influence of the transport rate on the interspace volume has been examined by determining the size of the interspace at different hydrostatic pressures with and without active transport. The latter situation was established by substituting choline for sodium in the outside bathing solution. When no transport takes place the interspaces are smaller than in the transport cases. When no hydrostatic pressure is applied the interspace system is totally collapsed in the absence of transport, while a measurably volume persists in the presence of sodium transport.

In the original version of the hypothesis for the development of the frog skin potential the ion pump was assumed to be a sodium potassium exchange pump, carrying one potassium ion into the cell for each sodium ion pumped out. The functioning of the model is, however, independent of the coupling ratio between sodium and potassium. The ratio 1:1 was chosen because the cation pumps in erythrocytes, nerve cells, and muscle fibres at that time were believed to operate with such a coupling ratio. Attempts to determine the coupling ratio of the sodium pump in frog skin have not been successful so far. No relation between the Na-transport and the uptake of ^{42}K has been found. The potassium exchange with the inside bathing solution seems hardly to be affected by changes in the sodium transport *(Cereijido* and *Curran [4])*. It is a question, however, whether a structure like that of the frog skin epithelium makes possible a meaningful determination of the Na/K coupling ratio. If we assume that the main part of the sodium transport takes place in the first living cell layer the interspace facing the surface of these cells must also be the site of

the Na/K exchange pump. If ^{42}K is added to the inside solution in order to determine the ratio, the K-ions passing through the long winding interspaces may have been in and out of several cells before they reach – if ever – the reacting cell layer. As potassium is present in low concentration in the *Ringer* solution and in high concentration in the cells, the system may act as an ion exchange column so that a ^{42}K-ion present at one end of the interspace system is more likely to enter a cell than to pass further on through the interspaces. Such an exchange resin effect would very much complicate determinations of the Na/K coupling ratio.

In some epithelia, like that of the gall bladder, the interspaces are so long and narrow that the transport of NaCl into the interspaces may build up a substantial concentration gradient of this electrolyte, a 'standing gradient', which may lead to an osmotically driven transport of water even between isotonic solutions *(Diamond [8])*. Such an effect is not present in the frog skin the water transport between isotonic solutions being less than $1\ \mu l/cm^2/h$.

4. *Shunt paths*

In the frog skin the interspaces are normally closed towards the outside solution by *zonulae occludentes*, the 'tight seals', between the cells of the first living cell layer. In gall-bladder epithelium and certain other epithelia, e.g. small intestine, these seals are somewhat leaky to water and small ions *(Diamond [8])*. Under certain conditions, however, even the frog skin can be transformed into a leaky epithelium. Thus if the osmotic pressure of the outside bathing solution is increased relative to that of the inside solution the potential across the skin decreases, and the skin becomes permeable to large molecules like sucrose *(Ussing [32])*. The hypertonicity of the outer medium has apparently created a shunt path.

Recently it has been possible to demonstrate directly that this shunt path is an intercellular pathway. If the solution bathing the outside of a skin contains Ba-ions and the inside bathing solution sulfate-ions a precipitate of $BaSO_4$ will be formed where the two ion species meet. The result of such an experiment was that the interspaces were filled with $BaSO_4$ crystals whereas no crystals could be seen in the epithelial cells. The phenomenon stands out very clearly when the outside medium is made hypertonic with urea, but even with *Ringer* on both sides of the skin crystals may develop after several hours *(Ussing [33])*.

The frog skin thus seems to possess a variable intercellular shunt path which together with the cellular shunt and the active sodium mechanism determines the level of the skin potential.

Similar considerations are valid for other epithelia. However, the relative importance of the shunts, the length of the interspaces with ensuing smaller or greater transport anomalies due to standing gradients may modify the net result of the active sodium-potassium exchange pump.

Although sodium pump systems quantitatively dominates the picture of ion transporting epithelia, sodium independent transport systems can be found in some organs. A well-known example is the transport of hydrogen ion and of chloride ions in the gastric mucosa *(Hogben [14])*. In insects (but probably not in vertebrates) a sodium independent potassium transport seems to play an important role *(Ramsay [28]*, *Harvey* and *Nedergaard [13])*.

Bibliography

1. *Baba, I.W., Lant, A.F., Smith, A.J., Townshend, Mary M.,* and *Wilson, G.M.:* Pharmacological effects in animals and normal human subjects of the diuretic amiloride (MK-870). Clin. Pharmacol. Therapeut. *9*, 318–327 (1968).
2. *Bentley, P.J.:* The effects of neurohypophyseal extracts on water transfer across the wall of the isolated urinary bladder of the toad *Bufo marinus*. J. Endocrinol. *17*, 201–209 (1958).
3. *Bentley, P.J.:* Amiloride: A potent inhibitor of sodium transport across the toad bladder. J. Physiol. *195*, 317–330 (1968).
4. *Cereijido, M.,* and *Curran, P.F.:* Intracellular electrical potentials in frog skin. J. Gen. Physiol. *48*, 543–557 (1965).
5. *Cereijido, M.,* and *Rotunno, C.A.:* Transport and distribution of sodium across frog skin. J. Physiol. *190*, 481–497 (1967).
6. *Civan, M.M., Kedem, O.,* and *Leaf, A.:* Effect of vasopressin on toad bladder under conditions of zero net sodium transport. Am. J. Physiol. *211*, 569–575 (1966).

7a. *Civan, M.M.,* and *Frazier, H.S.:* The site of the stimulatory action of vasopressin on sodium transport in toad bladder. J. Gen. Physiol. *51*, 589–605 (1968).

7b. *Comar, C.L.,* and *Bronner, F. (Eds):* Mineral Metabolism: an Advanced Treatise, 2 vols. New York: Academic Press, 1960.

8. *Diamond, J.M.:* The mechanism of solute transport by the gall bladder. J. Physiol. *161*, 474–502 (1962).
9. *Engbk, Lise,* and *Hoshiko, T.:* Electrical potential gradients through frog skin. Acta physiol. scand. *39*, 348–355 (1957).
10. *Farquhar, M.G.,* and *Palade, G.E.:* Functional organization of amphibian skin. Proc. Nat. Acad. Sci. *51*, 569–577 (1964).
11. *Frazier, H.S., Dempsey, E.F.,* and *Leaf, A.:* Movement of sodium across the mucosal surface of the isolated toad bladder and its modification by vasopressin. J. Gen. Physiol. *45*, 529–543 (1962).
12. *Fukuda, T.R.:* Über die Bedingungen für das Zustandekommen des Asymmetriepotentials der Froschhaut. Jap. J. med. Sc., Part III, Biophys. *8*, 123 (1942).
13. *Harvey, W.R.,* and *Nedergaard, Signe:* Sodium-independent active transport of potassium in the isolated midgut of the Cecropia silkworm. Proc. Nat. Acad. Sci. *51*, 757–765 (1964).
14. *Hogben, C.A.M.:* The chloride transport system of the gastric mucosa. Proc. Nat. Acad. Sci. *37*, 393–395 (1951).
15. *Keynes, R.D.:* From frog skin to sheep rumen: a survey of transport of salts and water across multicellular structures. Quart. Reviews of Biophysics *2*, 177–281 (1969).
16. *Koefoed-Johnson, V.:* Unpublished work quoted by *Ussing, H.H.,* in 'Ion Transport across Membranes' *(H.T. Clarke* and *D. Nachmansohn,* Eds.), p. 3. Academic Press, Inc., New York, 1954.
17. *Koefoed-Johnson, V.:* The effect of g-strophantin (ouabain) on the active transport of sodium through the isolated frog skin. Acta physiol. scand. *42*, suppl. 145, 87 (1957).
18. *Koefoed-Johnsen, V.:* Unpublished work quoted by *H.H. Ussing* and *E.E. Windhager* in 'Water and electrolyte metabolism' (II). *J. de Graeff* and *B. Leijnse,* Eds. Elsevier Publ. Comp., Amsterdam, 1964 a.
19. *Koefoed-Johnsen, V., Levi, H.,* and *Ussing, H.H.:* The mode of passage of chloride ions through the isolated frog skin. Acta physiol. scand. *25*, 150–163 (1952).
20. *Koefoed-Johnsen, V.,* and *Ussing, H.H.:* The contributions of difussion and flow to the passage of D_2O through living membranes. Effect of neurohypophyseal hormone on isolated anuran skin. Acta physiol. scand. *28*, 60–76 (1953).
21. *Koefoed-Johnsen, V.,* and *Ussing, H.H.:* The nature of the frog skin potential. Acta physiol. scand. *42*, 298–308 (1958).
22. *Koefoed-Johnsen, V.,* and *Ussing, H.H.:* Ion transport. In: Mineral metabolism. *1*, Part A. Academic Press, New York, 1960. Eds. C.L. Comar and Felix Bronner.
23. *Leaf, A.,* and Dempsey, E.: Some effects of mammalian neurohypophyseal hormones on metabolism and active transport of sodium by the isolated toad bladder. J. Biol. Chem. *235*, 2160–2163 (1960).
24. *MacKnight, A.D.C., Leaf, A.,* and *Civan, M.M.:* Vasopressin: Evidence for the cellular site of the induced permeability change. Biochim. Biophys. Acta *222*, 560–563 (1970).
25. *MacRobbie, E.A.C.,* and *Ussing H.H.:* Osmotic behaviour of the epithelial cells of frog skin. Acta physiol. scand. *53*, 348–365 (1961).
26. *Mirsky, A.E.,* and *Allfrey, V.:* The role of the cell nucleus in development. From: A Symposium on the Chemical Basis of Development. Eds. William D. McElroy and Bentley Glass. The John Hopkins Press, Baltimore 18, Maryland, 96–100, 1958.
27. *Nielsen, R.,* and *Tomlinson, R.W.S.:* The effect of amiloride on sodium transport in the normal and moulting frog skin. Acta physiol. scand. *79*, 238–243 (1970).
28. *Ramsay, J.A.:* Active transport of potassium by malpighian tubules of insects. J. Exptl. Biol. *30* 358 (1953).
29. *Stein, W.D.:* The movement of molecules across cell membranes. *6:* Theoretical and experimental biology. Academic Press, New York, 1967.
30. *Ussing, H.H.:* The active ion transport through the isolated frog skin in the light of tracer studies. Acta physiol. scand. *17*, 1–37 (1949) a.
31. *Ussing, H.H.:* The distinction by means of tracers between active transport and difussion. Acta physiol. scand. *19*, 43–56 (1949) b.

32. *Ussing, H.H.:* Anomalous transport of electrolytes and sucrose through the isolated frog skin induced by hypertonicity of the outside bathing solution. Ann. New York Acad. Sci. *137*, 543–555 (1966).
33. *Ussing, H.H.:* Tracer studies and membrane structure. From: Capillary permeability. The transfer of molecules and ions between capillary blood and tissue. Alfred Benzon Symp. II, Copenhagen June 1969. Eds. Chr. Crone and N. Lassen. Munksgaard, Copenhagen, 1970.
34. *Ussing, H.H.*, and *Zerahn, K.:* Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. Acta physiol. scand. *23*, 110–127 (1951).
35. *Ussing, H.H.*, and *Windhager, E.E.:* Active sodium transport at the cellular level. From: Water and electrolyte metabolism (II). J. de Graeff and B. Leijnse (Eds.). Elsevier Publ. Comp., Amsterdam, 1964 a.
36. *Ussing, H.H.*, and *Windhager, E.E.:* Nature of shunt path and active sodium transport path through frog skin epithelium. Acta physiol. scand. *61*, 484–504 (1964) b.)
37. *Ussing, H.H., Kruh ffer, P., Hess Thaysen, J.*, and *Thorn, N.S.:* The alkali metal ions in biology. Handbuch der experimentellen Pharmakologie, Band 13. Springer-Verlag, Eds.: O. Eichler and A. Farah, 1960.
38. *Voûte, C.L.*, and *Ussing H.H.:* Some morphological aspects of active sodium transport. The epithelium of the frog skin. J. Cell Biol. *36*, 625–638 (1968).
39. *Voûte, C.L.*, and *Ussing, H.H.:* The morphological aspects of shunt-path in the epithelium of the frog skin *(R. temporaria)*. Exp. Cell Res. *61*, 133–140 (1970) a.
40. *Voûte, C.L.*, and *Ussing, H.H.:* Quantitative relation between hydrostatic pressure gradient, extracellular volume and active sodium transport in the epithelium of the frog skin *(R. temporaria)*. Exp. Cell Res. *62*, 375–383 (1970) b.
41. *Zerahn, K.:* Oxygen consumption and active sodium transport in the isolated and short-circuited frog skin. Acta physiol. scand. *36*, 300–318 (1956).
42. *Zerahn, K.:* Nature and localization of the sodium pool during active transport in the isolated frog skin. Acta physiol. scand. *77*, 272–281 (1969).

Biological Rhythms of Potassium Metabolism*

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Summary: see at the end of this paper

1. *Introduction*

Biological rhythms of potassium metabolism can be demonstrated in man as well as in other animal [1–19, 24, 52, 69–72, 75, 95]. These rhythmical and predictable variations have been studied extensively a) in healthy human subjects under various experimental situations and/or conditions e.g. new-borns raised in self demand [*20, 21*]; adults following their usual daily routine of activity and rest, or desynchronized by environmental manipulations such as underground isolation without time cue [*9, 17*]; and in intercontinental flight across several time zones (transmeridian flight) [*13, 22, 95*] and b) in patients suffering from various diseases such as adrenal insufficiency – Addison disease – [*7, 23–26*], adrenal hyper-activity – Cushing syndrome – [*19, 27, 28*], etc.
Nowadays biological rhythms of potassium metabolism can be taken as an illustrative example of biorhythm analyses in man leading to a better knowledge of our biologic time structure. Apart from a contribution to basic sciences, parameters of potassium biorhythms can be used as rhythm references for other physiological rhythms; alterations in potassium metabolic rhythms related: a) to diseases and b) to some of the pharmacologic effects of drugs (such as corticosteroïds) are, *inter alia,* interesting indexes from the point of view of temporal pathology as well as temporal pharmacology.
To clarify this review it may be useful to summarize some specific advances in biorhythm research.
Biological rhythms can be defined as statistically validated physiologic changes recurring with a reproducible waveform. From a 'macroscopic' point of view, a rhythm implies regular periodicity of data displayed as a function of time. With the help of electronic computers and special programs – developed for this purpose at the *University of Minnesota* [*29–33*] – it is now possible to obtain a 'microscopic' characterization and quantification of rhythm. These methods of data analysis can reveal properties of time series with a power analogous to the resolving power of a good microscope [*33*]. From biologic time series a rhythm can be objectively detected ($P < 0.05$) and then can be characterized by the statistical estimation of several parameters: (average) period, τ,

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and/or (average) acrophase – crest time, ϕ, or crest angle, φ, – *amplitude* C and (average) *rhythm adjusted level* C_0. Endpoint and confidence interval estimates for these rhythm parameters are obtained by the use of approximating functions *inter alia*, by functions of the form:

$$y_{(t)} = C_o + C\cos(\omega t + \phi)$$

where ω = the angular frequency and t = time.

With reference to the period, τ (or to frequency: $1/\tau$), rhythms can be analysed, as a *spectrum* with statistically significant components in several spectral domains [*19, 29, 56*]. These are domains of *high frequency rhythms* ($\tau < 0.5$ hour), *medial frequency rhythms* (0.5 h. $< \tau <$ 2.5 days) and *low frequency rhythms* ($\tau > 2.5$ days).

No longer can one postulate that an animal organism is constant within the limits of a day, a month, or a year, etc.

Circadian rhythms (20 h. $\leq \tau \leq$ 28 h.) are included in the medial frequency domain. Most of the described cyclical changes in potassium metabolism have been circadian rhythms; therefore they will be the focus of this review.

Within certain limits, the period, amplitude and phase of circadian rhythms can be influenced by the cyclic variations of certain environmental factors. There are the alternation of day and night, heat and cold, noise and silence [*7, 12, 17, 19, 34–54*]. For man, the hours of work and repose, related to the duties of social life [*12, 15, 17, 19, 55–58*], comprise influences upon circadian rhythms. These factors are called *synchronizers* [*59*] *Zeitgebers* [*60*] or *entraining agents* [*61*] the three terms being synonymous.

Under the conditions of daily life, when the alternation of activity (in light) and rest (in darkness) is tied to a relatively regular schedule, the period of our circadian rhythms is, on the average, 24 hours. It will be the same for experimental animals in DL : 12/12. After certain manipulations of synchronizers (or of the organism) changes in phase and/or period can be observed. Nonetheless, synchronizers may influence ϕ, τ and C, but they do not cause rhythms. An organism's time structure as revealed by its rhythms can be considered a function of its genetic heritage.

For some species with their usual behavior and environment – and under certain experimental circumstances – some synchronizers can be called 'primary' or 'dominant'. Light-dark alternations are primary synchronizers for experimental animals [*11, 19, 34–54, 62*] and cyclical changes of socio-ecologic factors are primary for men [*12, 15, 17, 19, 55–58, 37, 95*]. This has strong implications for the methodology of research.

From a methodological point of view, any study on biological rhythms has to be realized under conditions standardized for periodicity [*34, 58*]. The following environmental conditions must be controlled: (1) lighting regimen for experimental animals, sleep-wakefulness schedule for human subjects; (2) duration and stability of the synchronization prior to the study. Without these criteria the analysis of data may be meaningless and it is possible to misinterpret the results.

Chronobiology is the study of the temporal characteristics of biologic phenomena, providing an objective description of biologic time structure [*19, 35*]. Biologic time structure, in turn, can be defined as the total sum of non random, and thus predictable, temporal aspects of organismic behavior. This includes bioperiodicity, rhythms of susceptibility, and developmental changes. Biologic time structure characterizes species and individuals as well as groups of organisms or their components: organ systems, organs, tissues, cells and intracellular elements (including ultramicroscopic structures). Rhythmi-

cal changes can be observed and demonstrated at all levels of organization. Thus rhythmical activity can be considered objectively as a fundamental property of living matter [*7, 17, 19, 36*].

Chronobiology includes a number of 'subspecialities', among them:

1. *Chronophysiology:* investigation of the temporal features of physiologic behavior and of physiological factors underlying biologic temporal characteristics.
2. *Chronotoxicology:* investigation of undesired or harmful consequences from chemical, physical, or other agents including poisons, pollutants and overdoses of drugs as they affect biologic temporal characteristics and as they act as functions of biologic timing.
3. *Chronopharmacology:* investigation of drug effects upon biologic temporal characteristics and drug effects as a function of biologic timing.
4. *Chronopathology:* investigation of alterations in biologic temporal characteristics as a function of disease and as determinants of disease.

Experiments in chronobiology have been selected for summary in this review on the following bases:

1. appropriate methodology for optimum control of synchronizers and data gathering; — 2. accurate technical procedures for determinations, measures, etc., on each biologic variable selected as an index of periodicity; and most important — 3. statistical analyses of time series data, by microscopic rather than macroscopic methods of parameter estimation. At the present stage of knowledge in chronobiology, a quantitative description of rhythmic phenomena should take priority over other problems such as the interpretation of observed rhythms.

2. *Circadian chronophysiology of potassium metabolism in the man*

2.1 *Healthy adults* (controlled usual routine of activity and repose. Spontaneous diet.)

Circadian variations in K^+ concentration have been detected in plasma, in cells (erythrocytes) and in urine.

Plasma potassium variations are of small amplitude [*66, 67*]. This explains the conclusions of several authors, from macroscopic examination of time series, that such rhythms do not exist. In fact, they can be detected objectively.

However the rhythm in erythrocytes and above all, in urines, is an easier way to study these variations. For this reason mainly, most of the investigations devoted to potassium rhythms in physiology, pharmacology and pathology are focused on urinary excretion.

Several parameters characterizing urinary circadian rhythms in potassium excretion are presented in Figure 1. Twelve healthy adults synchronized by L:0700–2300/ D:2300–0700* for at least 8 days collected their urines every 4-hours (Δt:4 h.) during 24 h.(T = 1 day). In these conditions the acrophase, $\varnothing$ (peak of the sine function used to approximate the rhythm) is found at 1344 (from 1200 to 1456 with 95% of security). Circadian amplitude C, expressed as per cent of deviation from mean is 44 (from 33 to 55).

* Clock hours are given according to international nomenclature in hours and minutes for 24 h. (i.e., 6 am = 0600; 6 p.m. and 37 min. = 1837; noon midday = 1200; noon midnight = 2400 or 0000).
Alternation of 12 h. of darkness, 12 h. of light can be written: DL: 12/12. Constant light = LL. Constant darkness = DD.

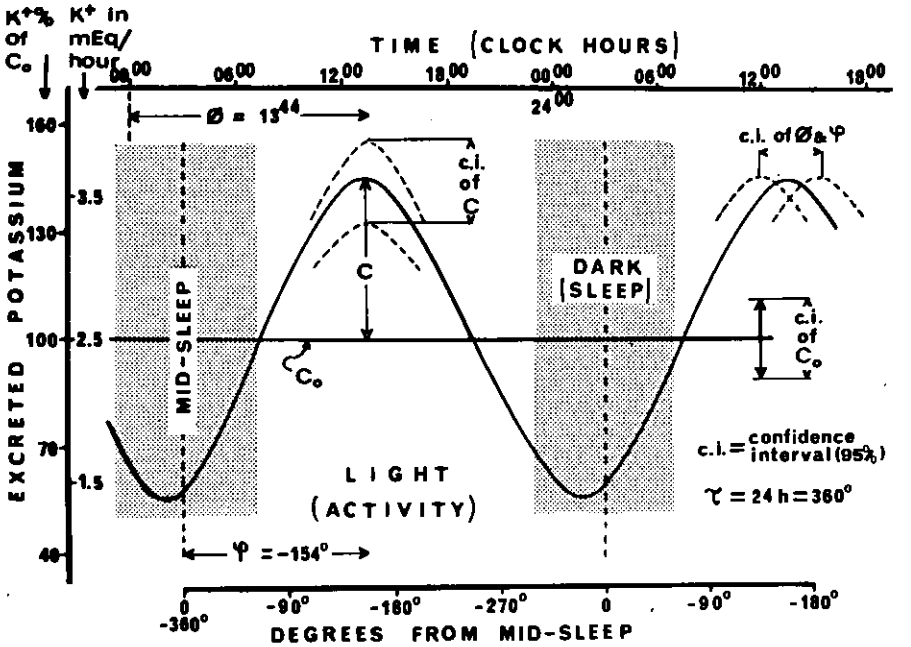

Figure 1. A biological rhythm can be assimilated to a sine function. Halberg's cosinor and related means of analysis are useful to find the best fitting sine function (least squares method); this leads to estimate several parameters which characterize objectively the rhythm: the period τ, the acrophase $\varnothing$ or φ, the amplitude, C, the rhythm adjusted level, Co etc. With the electronic computation, each one of these parameters is obtained with 95% confidence limits.
The circadian rhythm of K^+ urinary excretion is taken here as an illustrative example. Transverse study – 12 subjects – Synchronization (for at least one week before the study) with L (light): from 07^{00} to 23^{00} and D (dark): from 23^{00} to 07^{00}. Duration of each individual study: T = 24–48 h. Sampling interval: Δ t: 4 h. Spontaneous diet. The period τ is given here by the experimental conditions. As a mean $\tau = 24$ h = 360°. The acrophase (peak of the sine function used to approximate the rhythm) can be given in hours and minutes with midnight (00^{00} or 24^{00}) as an (arbitrary) phase reference. This computative acrophase $\varnothing = 13^{44}$ (95% C.I. 12^{00}–14^{56}). The acrophase can be given also in degrees ($\tau = 360°$) with midsleep as the phase reference of greater biological interest. This external acrophase $= -154°$ (from $-128°$ to $-172°$). The amplitude, C, can be expressed in mEq/h.: C = 1.10 (from 0.82 to 1.38) or expressed as percentile deviation from Co: C = 44 (from 33 to 55). In other words K^+ urinary excretion can be as high as 3.9 mEq/h. at the acrophase, and reach 1.1 mEq/h. 12 h. later or earlier for the choosen experimental example. (Data from *Reinberg, Zagula-Mally, Ghata,* and *Halberg* [76].)

Figure 2 shows a circadian aspect of human temporal structure including potassium variations in erythrocytes and urine. There is a time relationship between the circadian peak (acrophase) of the considered functions. Nervertheless time relationship does not mean necessarily direct causal relationship.

When healthy subjects are submitted to similar synchronization, *longitudinal study* (i.e. one subject collecting his urines for 21 days with Δ t ~ 4.5 h.) and *transverse study* (i.e. 7 subjects collecting their urines during 1 day with Δ t = 4 h.) lead to similar results (see Table 1).

Longitudinal as well as transverse studies have been done on various circadian rhythms by different investigators working independently in different cities and countries of the world.

Human Circadian System

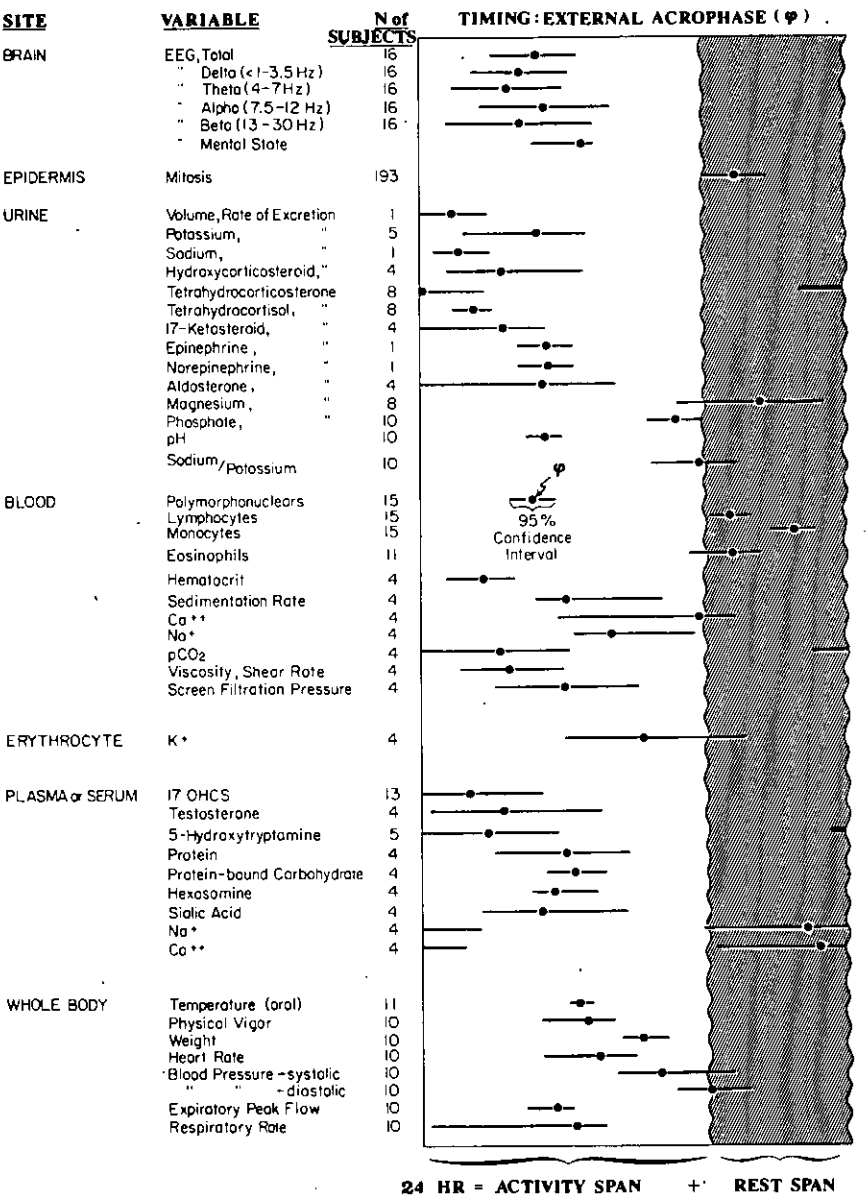

Figure 2. Aspect of human (healthy adults) circadian temporal structure. For each of the studied physiologic variables the acrophase φ is given with its 95% confidence limits. φ correspond to the peak of the cosine function used to approximate the rhytmical variation with *Halberg's* cosinor method. This circadian acrophase chart, shows timing in relation to light span, on top. Acrophase, φ, in relation to the rest-activity cycle, may roughly be approximated by reference to bottom scale. *(Halberg, Nelson, Doe, Bartter,* and *Reinberg* [74]).

Table 1. Circadian rhythm of several functions – Comparison between longitudinal and transverse studies

Variable investigated (site)	No of subjects (No of days) [Δt, h]	Noise to signal SE/C	P Rhythm Detection	Circadian Amplitude C (95% confidence limits)	Acrophase ϕ	Ref.
Oral temperature: °C						
(France)	1 (21) [2–10]	0.071	—	0.50 (0.42 to 0.58)	−185° (−174 to −196)	*[64]*
(France)	7 (1) [4]	—	<0.006	0.18 (0.07 to 0.29)	−183° (−135 to −219)	*[64]*
(Minnesota)	1 (39) [3–24]	0.167	—	0.18 (0.12 to 0.24)	−197° (−178 to −216)	*[64]*
(Minnesota)	11 (1) [1.5]	—	<0.005	0.27 (0.20 to 0.33)	−199° (−181 to −220)	*[64]*
Heart rate: beats/min.						
(France)	1 (21) [2–10]	0.111	—	9.3 (7.2 to 11.4)	−219° (−203 to −236)	*[64]*
(France)	7 (1) [4]	—	<0.002	7.7 (4.5 to 10.9)	−194° (−162 to −220)	*[64]*
Potassium: Urinary excr. meq/h						
(Minnesota)	1 (52) [3.3]	0.135	—	1.6 (1.1 to 2.2)	−171° (−156 to −186)	*[63]*
(Minnesota)	8 (1) [3]	—	<0.001	2.1 (1.4 to 2.8)	−157° (−130 to −181)	*[64]*
(France)	1 (21) [2–7]	0.111	—	1.2 (0.9 to 1.4)	−159° (−146 to −172)	*[64]*
(France)	7 (1) [4]	—	<0.001	1.1 (0.7 to 1.5)	−161° (−142 to −178)	*[65]*
17-hydroxycorticosteroid: ur. excr. mg/h						
(Minnesota)	1 (34) [4]	0.171	—	0.17 (0.13 to 0.20)	−130° (−117 to −144)	*[63]*
(France)	7 (1) [4]	—	<0.005	0.07 (0.03 to 0.11)	−134° (− 70 to −174)	*[65]*

Microscopic analyses of these time series are in good agreement, as it can be seen on Figure 3, for two physiological functions: urinary potassium and 17-hydroxycorticosteroïd excretion in healthy subjects with known synchronization.

All the results presented thus far reflect potassium metabolism in apparently healthy adults carrying on their usual activities during the day and following their usual diet unchanged in quantity, in quality as well as in timing of food intake.

2.2 *New-born*

A set of physiologic variables have been studied in new-borns raised in self-demand from first day of life to about 1 or 2 years [*17, 20, 21, 68*]. According to *Hellbrügge* [*20, 21*]: a) different physiologic functions develop a circadian rhythm independently; b) the circadian rhythm of the different functions becomes apparent at different times after birth; c) the development of circadian rhythm depens upon the maturity of the child at birth. The circadian periodicity develops later in premature than in children born at term. Daynight differences become statistically significant ($P < 0.01$) during the 1st week of life for electrical skin resistance, between the 2nd and the 3rd week for sleep wakefulness pattern, body temperature, between the 4th and the 20th week, for heart rate, urine volume, potassium and sodium excretion, between the 16th week and the 20th month, for phosphate and creatine excretion, etc.

Rhythmical activity can be demonstrated in new-born with a progressive shift from ultradian ($\tau < 20$ h) to circadian prominence. This possibility is suspected for potassium excretion but as yet is not demonstrated experimentally.

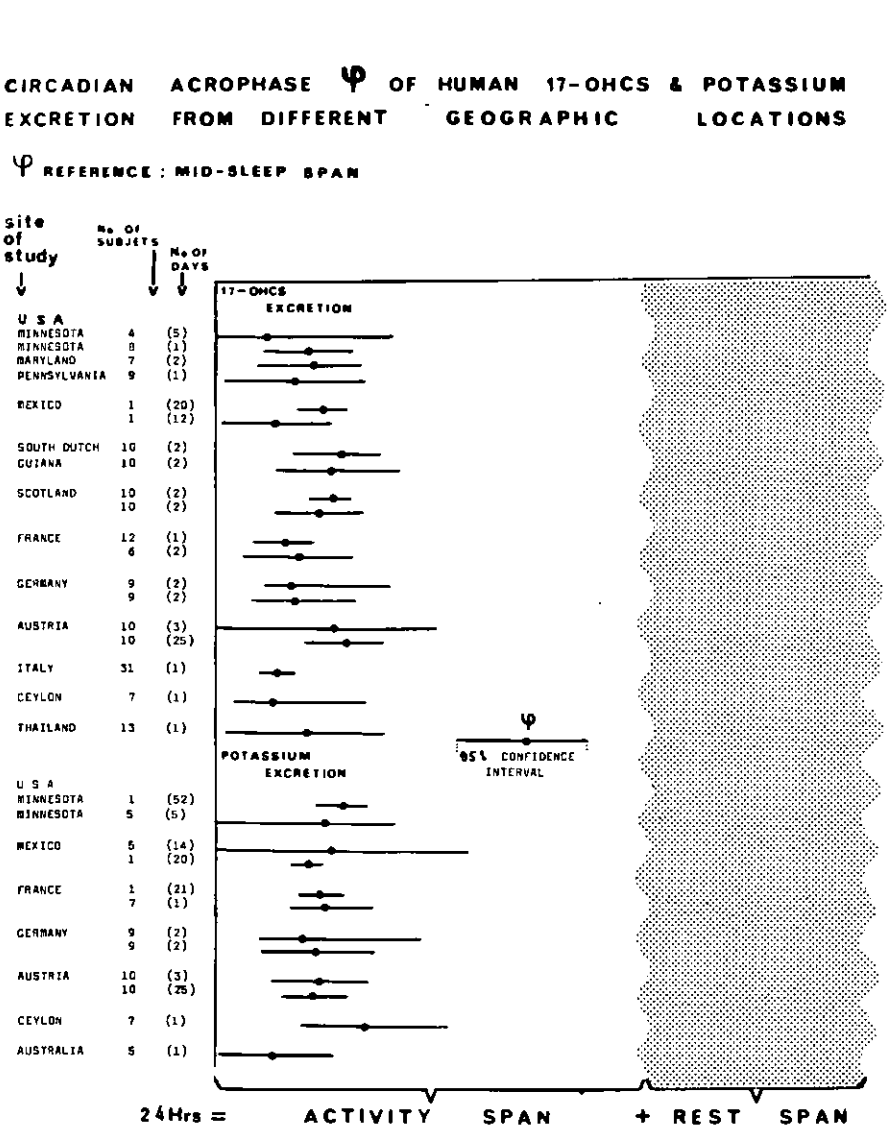

Figure 3. Circadian acrophase chart, showing timing in relation to light span, on top. Acrophase, φ, in relation to the rest-activity cycle, may roughly be approximated by reference to bottom scale. The figure reveals the extent of agreement in the timing of the circadian acrophases in 17-OHCS and potassium urinary excretion from healthy human beings living on different continents. *(Halberg, Reinhardt et al. [64]; Reinberg,* unpublished data).

2.3 *Manipulations of food and/or potassium intake*

Circadian variations of potassium metabolism can be detected in fasting subjects, during total deprivation of potassium intake, and in subjects receiving equal quantities of food and potassium at equal intervals.

Healthy adults were submitted [5] to different diets during 48 hours before and during the

study (T = 1 day, Δ t = 4 h.). A: neither potassium nor sodium intake; B: KCl 1 g, NaCl 3 g/24 h.; C: potassium overload ~ 8 g of KCl/24 h.; water and salts being given at equidistant intervals; Caloric intake was not controlled. Macroscopic examination of time series for potassium excretion [5] as well as microscopic analyses do not show alteration of phase and amplitude values. The level of the function (C_0) is the lowest with diet A and the highest with diet C.

Mills et al. [*69, 70, 71, 72*] reported circadian rhythms of potassium and sodium excretion in a subject continously engaged in laboratory work and taking small identical liquid meals every hour [*69*], in a subject remaining recumbent and fasting after waking in the morning [*72*] and on a 12-hour cycle of activity [*71*].

Potassium excretory rhythm among other circadian variations have been studied by *Reinberg et al.* [*65*] with the help of seven healthy adult volonteers on two regimens: a) habitual diurnal activity and uncontrolled diet and b) complete 36-hour bedrest with 4-hourly hypocaloric meals during sampling (K^+: 42 meq/24 h. — glucose 84 g/24 h.). Acrophases and amplitudes of the studied rhythms do not show any statistically significant difference between a and b (Figure 4).

Apfelbaum, Reinberg et al. (unpublished data) did not find any statistically significant changes in urinary circadian rhythms of K, 17-OHCS, 17-KS in 16 healthy obese young women before and after 2 to 3 weeks of caloric restriction and loss of ~ 10 kg of body weight. (The controlled diet was: 220 cal/24 h. as protein exclusively, and K: 20 mEq/24 h.).

Thus *food intake* (spontaneous diet or food restricted to 220 cal/24 h. as proteins as well as to 336 cal./24, as glucose), *potassium intake* (from 0 to ~ 140 meq/24 h.) bedrest, etc. do not detectably alter the acrophase and the amplitude of potassium circadian excretory rhythms among others of healthy synchronized subjects (with L: from ~ 0700 to ~ 2300 and D from ~ 2300 to ~ 0700).

2.4 *Suppression of known synchronizers*

Circadian components in the spectrum of rhythms characterizing sleep/wakefulness, rectal temperature and the excretion of K^+ and 17-hydroxycorticosteroids were evaluated in two healthy adults, a man and a woman, a) in the absence of known synchronizers during 3 and 4 months isolation in caves without time cues, and, b) under conditions of synchronization by societal cycles [*17, 37, 56, 57, 73*]. Time series of the above variables were analyzed by special electronic computer techniques.

For the case of isolation, such analyses suggest that the circadian rhythms in urinary K^+ and 17-hydroxycorticosteroid excretion, in rectal temperature and in sleep: clock while:

1) Persist in the absence of known synchronizers;

2) Are desynchronized with statistical significance from local 24-hour clock time; while:

3) Certain phase relations among the rhythms themselves are maintained. In isolation, just as it does under conditions of societal synchronization, the circadian acrophase of 17-hydroxycorticosteroid excretion continues to preceed the rectal temperature acrophase (within about a quarter period). Such a finding indicates that this aspect of the human time structure persists in the absence of environmental synchronization.

In subjects exposed to societal cycles, the influence of synchronizers manifests itself in a modification primarily of the average period, in that the 24-hour cyclic societal routine can impose its cycle length upon the rhythms. Findings in the present study indeed demon-

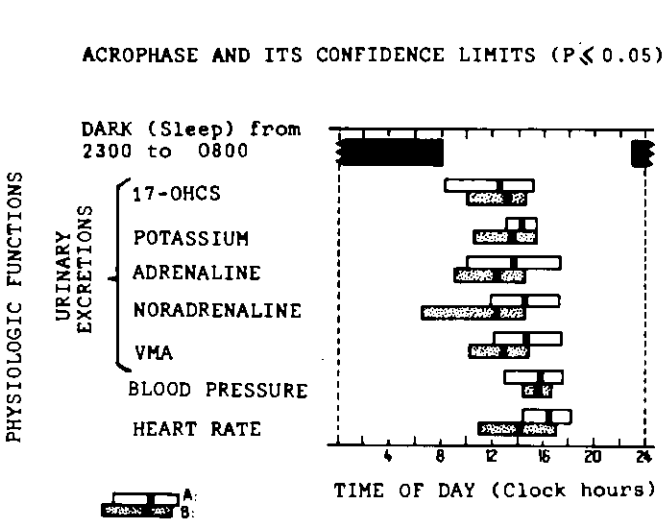

Figure 4. Circadian rhythmic heart rate, blood pressure and urinary potassium, 17-hydroxycorticosteroid and catecholamine excretion of healthy human adults during usual routine and bedrest with 4-hourly hypocaloric diet.

Acrophase, amplitude and corresponding 95% confidence limits were estimated on several circadian rhythmic variables of seven healthy adult volunteers on two regimens: a) habitual diurnal activity and uncontrolled diet, or b) complete 36-hour bedrest with 4-hourly hypocaloric meals during sampling (336 cal., 42 mEq of potassium/24 h.). On both regimens, subjects were in darkness from 23^{00} to 08^{00}, most of these hours being spent in uninterrupted sleep before the study began. (L: from 08^{00} to 23^{00}, D: from 23^{00} to 08^{00}, for at least one week before the study).

Heart rate and systolic blood pressure were measured and urines were collected starting at 07^{00} on a Sunday and continuing every 4 hours for 24 hours. The series were analyzed by electronic computer method, including the cosinor procedure; a statistically significant rhythm was detected for all variables under both conditions. Since complete bedrest over a span of 36 hours and an equidistant hypocaloric diet do not lead to their immediate disappearance, circadian rhythms in the urinary excretion of K^+, 17-OHCS, adrenaline, noradrenaline and vanilmandelic acid and in heart rate or blood pressure are not directly dependent upon cycles of rest and activity. (From *Reinberg, Ghata, Halberg, Gervais, Abulker, Dupont,* and *Gaudeau,* [65]).

strate resynchronization after prior spans of isolation associated with desynchronized rhtyhms.

We have to precise that, under conditions of study, a circadian peak occurs at $\tau = 24.6$ h. for the woman and at $\tau = 24.8$ h. for the man, both of these being statistically different from exactly 24 h. Spectral analyses of the considered time series for K^+ excretion show also additional prominent peaks in other spectral domains than circadian.

The results as a whole provide further evidence to suggest that 1) circadian rhythms (e.g. K^+ among others) depends, at least in part, upon factors that characterize the biologic system rather than its environment and that 2) environmental factors play, primarily, the role of a synchronizer.

2.5 *Phase shift of synchronizers* (Problems of transmeridian flights, of night-working and of shift-working)

The circadian system of healthy man is readily amenable to phase-shifting as a result of night-work or of intercontinental flights across several (at least 5) time zones – transmeridian flight (Figure 5). In such circumstances a phase-shift of socio-ecological synchronizing factors takes place without change in the synchronizing period ($\tau = 24$ h. as a mean).

Studies on 'dyschronism' (alteration of temporal structure) occuring after transmeridian flights [*13, 15, 19, 22, 75, 77, 95*] lead to the following remarks: 1) There will be a lag between a synchronizer phase-shift and corresponding shifts in the circadian rhythm acrophase of the physiologic variables studied. The time required to phase-shift the latter will vary according to the physiologic variable and to the subject and may range from a few days to several weeks.

2) This 'acclimation time' [*81*] after a transmeridian flight: a) varies from subject to subject for a given physiologic function; b) varies from function to function for a given subject (Figure 5); c) varies for a given function with direction of flight (being shorter after an East to West flight than after a West to East flight) (Figure 5).

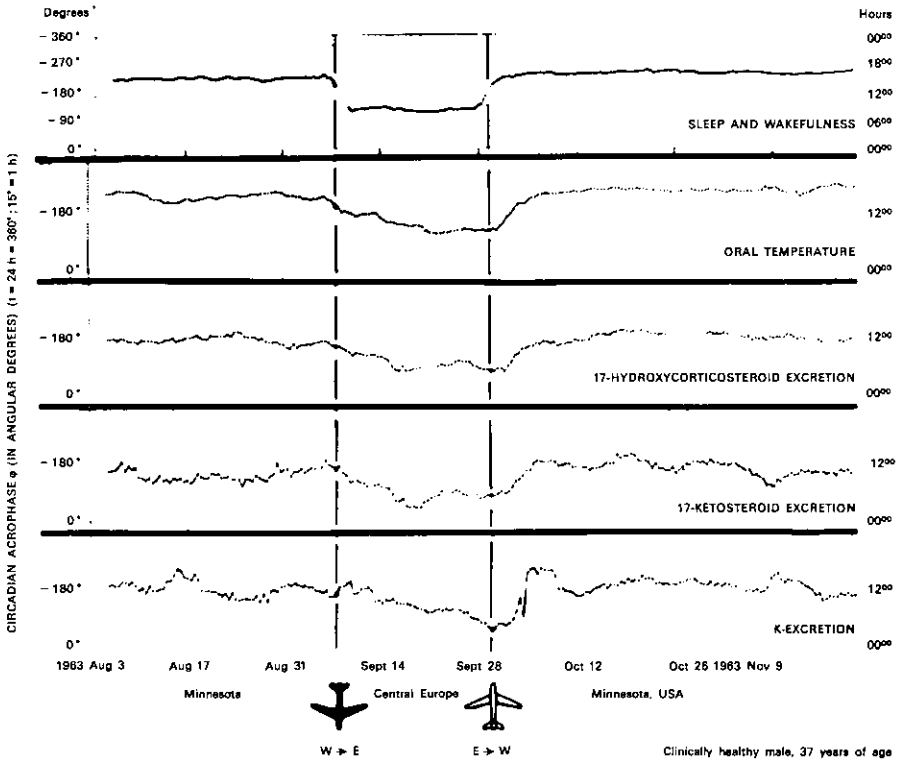

Figure 5. Two phase-shifts of human cardian system, following Intercontinental Flights (Minnesota, U.S.A., to Central Europe for 22 days and return to Minnesota).

A complete phase-shift of urinary K^+ circadian acrophase takes about 1 to 2 weeks [*19, 22, 75*], while it takes about 2 to 5 days for sleep-wakefulness rhythm and about 2 to 3 weeks for 17-OHCS circadian urinary excretion (Figure 5).
Continuous working at odd hours (i.e. habitual night work) results in acclimation of circadian rhythms, e.g. potassium among others [*3, 78*]. The circadian system of man usually working at night in Paris (phase shift of about 12 h. in work-rest timing) ressembles in some instance, that of a night-sleeping and day-active subject living at the antipodes; both are desynchronized by about 12 h. in comparison with the night-sleeping and day-active subject in Paris.
From a chronobiologic point of view schedule of work with frequent shifts (i.e. weekly) or repeated and irregular shifts do not favor the synchronization of certain circadian rhythms (i.e. K^+ and 17-OHCS excretory rhythms) which usually take more than a week to phase-shift their acrophase.
As far as K^+ excretory rhythm a) is relatively easy to study; b) can be considered as a circadian index reflecting the kidney and the adrenal time structure (see interpretation of potassium rhythm), it could be interesting to select this physiologic variable when problems related to shift-working are concerned. This could help an understanding of the relation between diseases and alterations of time structure resulting from certain shift-working systems.

2.6 *Urinary near-24-hour rhythms in subjects living on a 21-hour routine in the arctic*

In addition to a single shift and to a suppression of known synchronizers it was pertinent to investigate the repeated synchronizer alterations which will occur if a routine is not precisely 24-hour periodic. The results of instituting an artificial 21-hour 'day/night cycle' in the continuous light of the arctic summer [*79, 80, 81*] for subjects accustumed to a 24 hour cyclic routine are useful and interesting to consider. Time series corresponding to longitudinal studies performed by *Simpson* [*79*], *Simpson* and *Lobban* [*80*], were reanalyzed microscopically by these authors and *Halberg* [*81*]. The occurence of beats in data from human beings living on a 21-hour day led *Halberg* [*12, 19*] to postulate a near 24-hour rhythm as an effect superimposed upon the artificial 21-hour input.
Eight human adults, living up to 7 weeks on a 21-h. regimen and collecting 18 subject-weeks of observation (1296 urine collections) provide no inferential statistical evidence for a progressive acclimation of the circadian 17-OHCS rhythm to the artificial circadian routine. For several variables the amplitude of the 21-h. effect represents a personal characteristic seemingly unaffected by increased experience of a 21-h. day.
Evidence was found to suggest that the desynchronized component does not have a period of precisely 24.0 h.; on the average it was slightly longer than 24 h.
The relative contribution of a 21-h. cyclic input into the human body to that organism's near 24-h. output can be assessed by a 'circadian amplitude ratio' (CAR). It may be computed from selected paired amplitude from the least square window.
The C at a 21-h. trial period, corresponding in this case to the social 'input' is divided into the C at $\tau = 24.2$. The latter spectral crest correspond to a presumably organismic contribution to the output. The CAR will roughly approximate the relative contributions of organism and environment to circadian periodic behaviour on an abnormal schedule. 'Nature's' contribution predominates over that 'nurture' when the CAR is larger than unity, and vice versa.

CAR's suggest (Figure 6) that in the case of 17-OHCS and potassium a presumably largely intrinsic spectral component has, on the average, at least twice the amplitude of the social 21-h. cycle effect.
By contrast, the 21-h. social routine is relatively important in generating circadian changes in urine pH, the CAR being 0.37.
A CAR ratio near unity in sodium, chloride, water and in sodium/potassium ratio is in keeping with nearly equal contributions of presumably intrinsic and extrinsic factors.

3. *Circannual rhythm in potassium metabolism*

From macroscopic examination of data collected in 1952–1954, on urinary volume and potassium excretion, *Ghata* and *Reinberg* [6] reported that a circannual rhythm seemed to occur. These time series have been microscopicaly reanalyzed for a well documented discussion about circannual rhythms in mice (see *Haus* and *Halberg* [82]).
Circannual rhythms may be expressed, among others, as changes in level and/or in

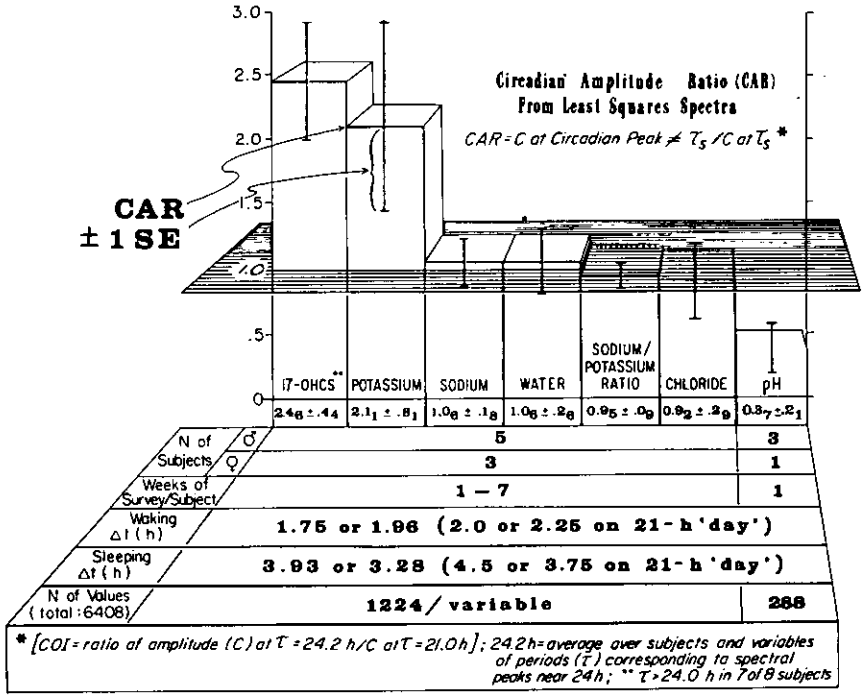

Figure 6. Amplitude ratios of the 24-hour frequency to the 21-hour frequency drived from least squares spectrum analysis. For 17-OHCS and potassium the 24-hour frequency had twice the amplitude of the 21-hour frequency. In contrast, pH showed mainly the 21-hour rhythm.

parameters (ϕ or C) of each of the rhythms in the several frequency domains so characteristic of the organism's biologic time structure Our data [6] suggest the occurence of a circannual shift in circadian acrophase of urinary volume and K^+ excretion by human subjects. A circadian acrophase was computed by the least squares fit of a 24-h. cosine curve to data on 6–8 subjects, each sampled at 4-h. intervals over a 24-h. span during either September or October in Paris (1952, 1954) and Copenhagen (1953), or during March-April (1954) in Paris. In order to focus upon possible circannual changes in circadian acrophase, the site where the profiles were obtained can be ignored. In ignoring further the circular distribution (in view of the location away from 360° of the acrophase and their proximity one to another), an F-test was then used to compare the 3 series obtained in September-October with that obtained in March-April. A statistically significant difference (below the 5% level) was thus detected. The circadian acrophase (with their standard errors) expressed as delay from local 00^{00} were located at -189° ± 8° for urine volume and at −184° ± 6° for urinary potassium excretion during September-October. The corresponding values for March-April were located at −261° and at −233°, respectively.

4. *Examples of circadian chronopathologic alterations in human potassium excretion*

From a macroscopic point of view alterations of circadian rhythm in water, K^+ and Na^+ excretion have been reported in several diseases such as congestive heart failure, cirrhosis, kidney disorders, adrenal dysfunctions, etc. [*7, 10, 23, 24, 25, 27, 39, 75, 83, 84*].
As illustrative examples adrenal insufficiency and adrenal hyperfunction have been selected because a) adrenal secretion and K^+ metabolism are closely related, and b) objective analyses of time series are available for these two endocrine diseases.
Alterations of the K^+ excretory circadian rhythm have been described, as a group phenomenon, in patients suffering from primary adrenal insufficiency (Addison's disease) or secondary to pituitary insufficiency [*5, 25, 86*]. Macroscopic examination (Figure 7) as

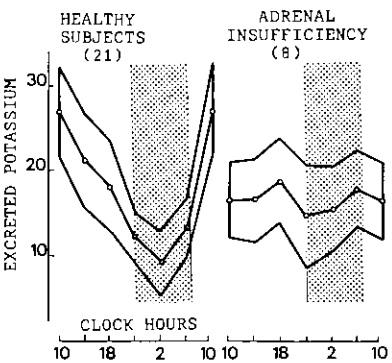

Figure 7. Circadian changes in K^+ urinary excretion in 21 healthy adults (left) and of 8 patients suffering from adrenal insufficiency (right). (T = 24 h.; Δ t = 4 h.; L from ~ 07^{00} to ~ 22^{00}, D from ~ 22^{00} to ~ 07^{00}; spontaneous diet). 4-hourly excreted K^+ is expressed as p. cent of total excreted K^+ for 24 h. Confidence limits are given for 95% security. As group phenomenon K^+ excretory rhythm is altered in patients suffering from adrenal insufficiency. *(Azerad, Ghata, Reinberg [23]; and Reinberg [86]).*

well as microscopic analyses of time series in transverse study show that K^+ circadian excretory rhythm cannot be detected. Alteration of several circadian rhythms (e.g. circulating blood eosinophils: *Halberg et al.* [87] have been described objectively in human adrenal insufficiency as well as in adrenalectomized mice *(Halberg [12, 88]*. For certain patients, alteration of urinary K^+ circadian rhythm is not found [*75, 85*]. Several explanations can be proposed for these alterations such as: variations in adrenal insufficiency, difficulties of interpretation in short individual time series, etc. Thus, one must point out that alteration of K^+ excretory circadian rhythm (among others) in untreated adrenal insufficiency is detected as a group phenomenon.

Cushing's syndrome corresponds to a pathologic hyperactivity of the adrenal secretions. Alterations of rhythm (or dyschronism) have been demonstrated objectively by *Halberg et al.* [*74*] and *Doe* [*89*] as a group phenomenon, in five patients suffering from this disease for the following variables: plasma hydroxycorticosteroid, urinary 17-OHCS, K^+, Na^+ and Mg^{++} (see Table 2).

Thus, the alteration of K^+ metabolism (among others) as a result of adrenal dysfunction has to be considered not only from the point of view of classical pathology but also as an example of chronopathological trouble in the circadian time structure of the organism.

Table 2. Circadian rhythmometry detects dyschronism in patients with Cushing's syndrome (*)

Group studied (N of subjects)	P; Cosinor (P; SE/C)	Mean, X or level, $C_o \pm SE$	Amplitude, $C \pm SE$ or (.95 confidence limits)	Acrophase, ϕ
		Plasma-hydroxycorticosteroid (μg/100 ml)		
Healthy (13)	<.005	13.5 ± .9	4.1 (2.3 to 6.0)	−100° (− 63 to −137)
Healthy (8)	<.002	7.4 ± .9	5.3 (2.7 to 7.9)	−113° (− 61 to −151)
Cushing's Syn. (5)	>.10	33.6 ± 5.7	3.0	− 64°
[Cushing's Syn. (5)	(>.10)	34.1 ± 2.2	2.7 ± 3.5	− 75°
		Urinary 17-hydroxycorticosteroid (mg/hr)		
Healthy (4)	<.03	.35 ± .04	.15 (.04 to .26)	−129° (− 83 to −200)
Healthy (8)	<.05	.35 ± .02	.12 (.06 to .17)	−153° (−128 to −177)
Cushing's Syn. (5)	>.10	1.23 ± .13	.06	− 11°
[Cushing's Syn. (5)	(>.10)	1.23 ± .06	.05 ± .02	−343°
		Urinary potassium (mEq/hr)		
Healthy (5)	<.005	3.2 ± .2	1.3 (.7 to 1.9)	−161° (− 98 to −202)
Healthy (8)	<.001	3.4 ± .2	2.1 (1.4 to 2.8)	−157° (−130 to −181)
Cushing's Syn. (5)	>.10	3.2 ± .4	.6	− 38°
[Cushing's Syn. (5)	(>.07)	3.1 ± .2	.6 ± .60	− 6°
		Urinary sodium (mEq/hr)		
Healthy (5)	<.02	9.4 ± 1.6	2.6 (.9 to 4.3)	−200° (−135 to −250)
Healthy (8)	<.02	5.4 ± .5	2.4 (.6 to 4.1)	−186° (−169 to −244)
Cushing's Syn. (5)	>.10	4.8 ± 1.3	.9	− 54°
[Cushing's Syn. (5)	(>.10)	4.5 ± .3	.9	− 31°
		Urinary magnesium (mEq/hr)		
Healthy (8)	<.04	.47 ± .04	.08 (.01 to .16)	− 3° (−294 to − 58)
Cushing's Syn. (5)	>.10	.40 ± .05	.04	−332°
[Cushing's Syn. (5)	(>.10)	.39 ± .06	.04	−310°

Acrophase reference = middle of daily sleep span (average 21^{00}–06^{30}).

(*) Rhythm detection carried out by the fit of a 24-hour cosine curve followed by cosinor summary of the paired (C, ϕ) values thus obtained for each individual series. (In last row for each variable, a fit to the mean values of the five patients with Cushing's Syndrome also is summarized and found to agree well with cosinor result.) No circadian rhythm is detected for the patients with Cushing's Syndrome *(Halberg et al. [74]: Doe [89])*.

5. *Examples of circadian chronopharmacological changes in human potassium excretion*

The possibility to induce a shift of circadian acrophase of certain rhythm by timing of drug administration, is now well documented [*90*].

Cortisol and corticosteroids are able to induce a shift of circadian acrophase in K^+ excretion. Two examples can be given to illustrate this fact.

Circadian rhythms in peak expiratory flow rate (PEFR) and in urinary excretion of potassium and chloride are detectable in healthy children as well as in children suffering from asthma. *Reindl et al.* [*92*] and *Halberg* [*93*] have shown that prednisone treatment did not act similarly when the drug was given at different phases of the circadian system to children with severe steroid-dependant asthma. The different fixed times of drug administration were 01^{00}, 07^{00}, 13^{00} or 19^{00}. When prednisone was given daily at 07^{00}, i.e. at (or near) the acrophase of the endogenous plasma 17-OHCS rhythm, the K^+ excretory acrophase remained roughly unchanged (Figure 8) as well as PEFR acrophase. Comparable doses of the identical drug given at other circadian system phases induced a statistically significant shift in the acrophases of the studied rhythms. This effect consisted of a statistically significant delay when the drug was given at 13^{00} daily. By contrast, an advance of acrophase was recorded when the drug was given daily at 01^{00} or at 19^{00} (Figure 8).

The other example is concerned with a study by *Reinberg et al.* [*26, Figure 9*] of 'timed' treatment in adrenal insufficiency. The circadian rhythm of rectal temperature, heart rate, grip strength and urinary excretion of 17-hydroxy corticosteroids, 17-ketosteroids, potassium and sodium were assessed in seven healthy adults and in seven adult patients with an adrenal cortical insufficiency secondary to hypophysectomy for removal of adenoma (two patients) or as result of Addisons's disease (five others). Similarly synchronized subjects were sampled at 4-hourly intervals for 24 or 48 hours. Oral corticosteroid treatment (Cortisol, 25–35 mg/24 h., with or without 50 μ g/24 h. of 9-α-fluorocortisol) was given in one of two ways to the patients with adrenal insufficiency. Mode A: three equal medications at 08^{00}, 13^{00} and 20^{00} (mealtimes). Mode B: two unequal doses, ¾ or ⅔ of the total dose at 07^{00} (at awakening or on getting up) and the rest at 23^{00} (upon retiring). Four of the patients were studied on both medication modes.

For all groups, healthy subjects and patients with adrenal insufficiency treated according to Mode A or B, statistically significant rhythms were detected. Differences in amplitude or acrophase were not detected for rectal temperature and heart rate in the patients, as compared to healthy subjects. Circadian rhythms in grip strength and urinary excretion of 17-OHCS, 17-KS; K^+ and Na^+ in patients treated according to Mode A are delayed in acrophase by about 6 hours, as compared to controls. By contrast, the acrophase and the amplitude of the same functions did not differ detectably between the healthy subjects and those patients treated according to Mode B. The physiologic results are in keeping with a suggestion that in the treatment of adrenal insufficiency the daily dose of corticoids be distributed in such a fashion that ⅔ or ¾ of it are given on awakening and the rest before retiring.

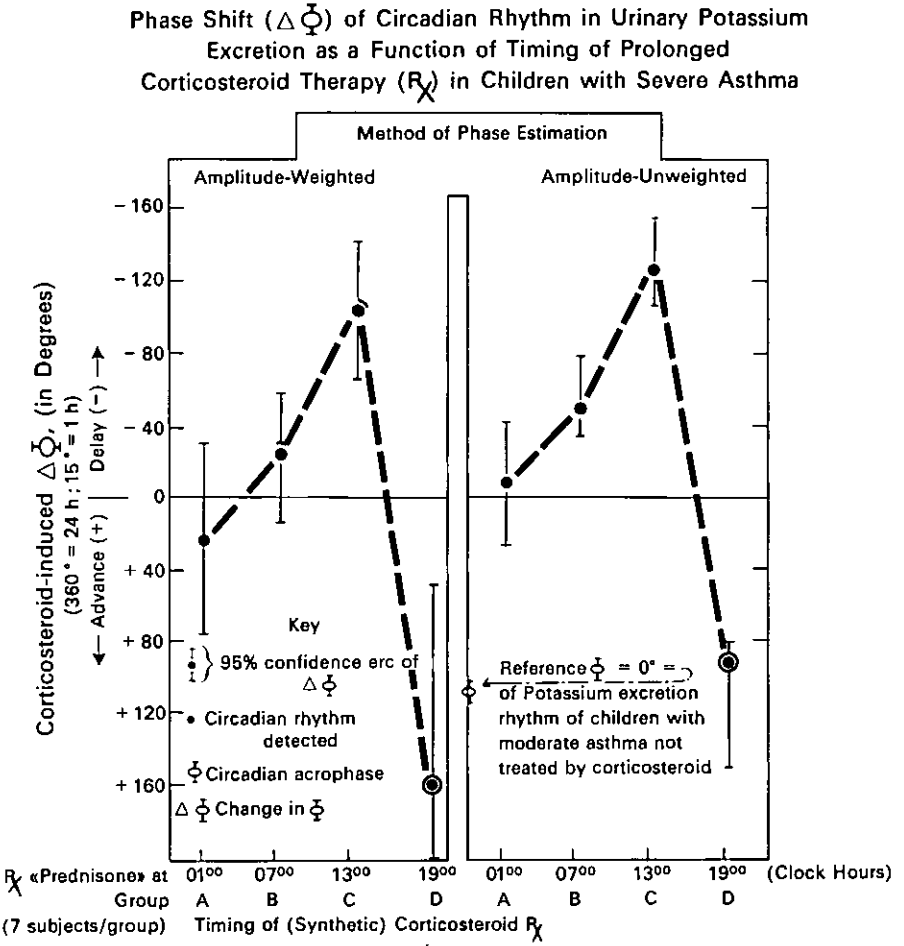

Figure 8. Drug-induced shift of circadian acrophase in urinary potassium excretion; also drastically different effects as a function of the timing of medication. *(Reindl, Falliers, Halberg, Chai, Hillman, Nelson* [92]*; Halberg* [93]*).*

6. *Interpretation of potassium excretory rhythm. Its practical applications*

From all the experimental facts summarized in this review it appears that potassium excretory is a well documented example of an intrinsic rhythm in man. Several of its parameters can be changed by the manipulation of synchronizers, nevertheless synchronizers do not create the rhythm.

Several temptatives have been done to find a specific relationship between potassium rhythm and a physiologic function *(Conroy* and *Mills* [75]*).*

Obviously, the kidneys, the adrenal glands and other organs or tissues with rhythmic activity play a role to induce K^+ excretory rhythm; but no one of these organs or tissues alone can be considered as the clock or as the inducer of this rhythm.

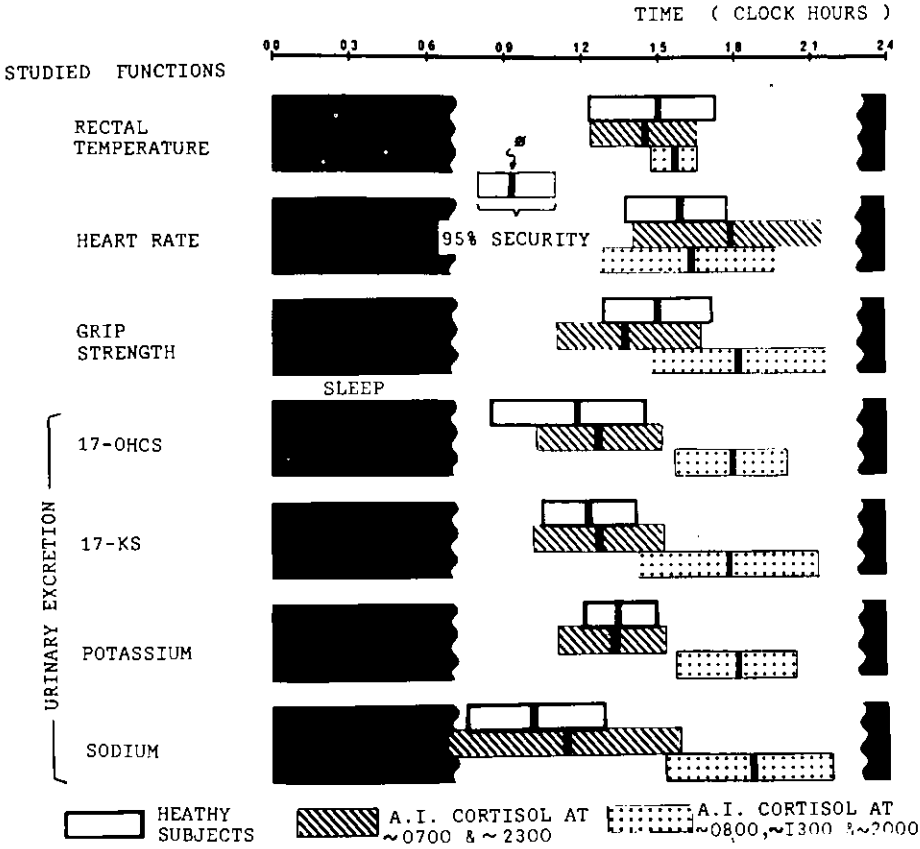

Figure 9. Circadian acrophase of a set of physiologic variables in healthy subjects and in patients suffering from adrenal insufficiency (A.I.) when receiving cortisol at different times (T=24—48 h.; Δ t: 4 h.; L from 07^{00} to 23^{00}, D from 23^{00} to 07^{00}, spontaneous diet).
Circadian rhythms in grip strength and urinary excretion of 17-OHCS, 17-KS, K^+ and Na^+ in patients treated with 3 equal doses of cortisol at 08^{00}, 13^{00} and 20^{00} are delayed in acrophase by about 6 hours, as compared with controls. By contrast, the acrophase of the same functions did not differ detectably between healthy subjects and patients treated 2/3 or 3/4 of Cortisol daily dose at 07^{00} and the remaining fraction at 23^{00}. *(Reinberg, Ghata, Halberg, Apfelbaum, Gervais, Boudon, Abulker, Dupont [94]).*

We know that, on the one hand, biorhythms can be demonstrated at all level of organization, including molecular level, since the rhythmical activity is a fundamental property of the living matter; on the other hand, the temporal structure leads us to consider a rhythmical physiologic variation as the result of several component variations taking place at various levels of organization. Therefore potassium excretory rhythm is likely to be the result of several component cyclic variations involving rhythmical activities in adrenals, kidneys, nervous system, etc. Even if the adrenal seems to be a very important factor in sodium and potassium excretory rhythms, other factors have not to be neglected. In addition, rhythms of adrenal secretions are themselves associated with adrenal activity

rhythms such as circadian variations in mitoses, metabolic processes, response to ACTH stimulation, etc. [*15, 17, 19, 93*] which can be considered also as component rhythms. A better interpretation of the K^+ metabolic rhythm will become possible when more objective studies will be published. Rhythm quantification is the first problem to be solved in this context. Such consideration has been forgotten many times leading to opposite interpretations and misunderstandings between authors.

From a biological point of view, potassium rhythms cannot be neglected or ignored in studies devoted to potassium metabolism, or in which potassium changes are involved. This important practical aspect has been recognized by several authors since the early fifties [*2, 4, 5, 23, 24*].

From a chronobiologic point of view, the K^+ excretory rhythm – as well as body temperature rhythm – can be considered as a reference circadian rhythm in the study of human temporal structure [*76, 94*]. Both are easy to obtain and well documented. Urinary potassium acrophase is one of the good indexes of circadian synchronization (or dysynchronization) and it can be used as phase reference.

7. *Summary and Conclusions*

Potassium metabolism, as reflected by plasma level, erythrocyte content and mainly urinary excretion, is not constant but varies rhythmically and predictably. Circadian – about 24 h. – rhythms have been demonstrated and studied in healthy man under a wide variety of experimental conditions.

Inferential statistical analyses – done with the help of special computer programs *(F. Halberg)* – characterize rhythms by defining several of their parameters; the period, τ, the acrophase, $\varnothing$, the amplitude, C, the rhythm adjusted level, C_0, etc. Each one of these parameter is given with its confidence limits for a desired degree of security, usually 95%. These 'microscopic' methods are the best we have to detect rhythms ($p < 0.05$) and to analyse objectively changes or alteration in certain experimental or pathological circumstances. Results presented in this paper were selected on the following basis: They were based on experimental studies far from major methodological criticism; the conclusions were founded on objective analyses of time series, and data of historical interest were also included.

The human circadian rhythm of K^+ urinary excretion has been detected and studied in healthy new-borns, in healthy adults on various diets and K^+ intake, and/or various type of activities. The rhythm persists, with a change of period (and amplitude) during isolation underground (without time cue) and also during a 21-hour routine. Its acrophase can be shifted by manipulation of synchronizing factors (i.e. shift-working, transmeridian flight) or by timed administration of drug such as corticosteroids. Alteration of K^+ excretory rhythm is interesting to consider in certain diseases: adrenal insufficiency, Cushing's syndrome, etc.

A circannual – about one year – rhythm in potassium excretion has been demonstrated, with a shift of circadian acrophase.

Such cyclical and predictable variations have to be taken into consideration in studies devoted to potassium metabolism or in which potassium changes are concerned.

Parameters of circadian potassium excretory rhythm (and specially its acrophase) can be selected as reference system in the study of human temporal structure.

Acknowledgments

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Bibliography

1. *Simpson, G.E.:* Changes in composition of urine brought about by sleep and other factors. J. Biol. Chem. *84*, 393 (1929).
2. *Stanbury, S.W.*, and *Thomson, A.E.:* Diurnal variations in electrolytes excretion. Clin. Sc. *10*, 267 (1951).
3. *Norn, M.:* Über Schwankungen der Kalium Natrium und Chlorid-Ausscheidung durch die Niere im Laufe des Tages. Skand. Arch. Physiol. *55*, 184–210 (1929).
4. *Fourman, P., Reifenstein, E.C., Kepler, E.J., Dempsey, E., Bartter, F.*, and *Albright, F.:* Effect of deoxycorticosterone acetate on electrolyte metabolism in a normal man. Metabolism, *1*, 242 (1952).
5. *Azerad, E., Lestradet, H., Reinberg, A.*, and *Ghata, J.:* Variations nycthémérales de l'élimination urinaire du potassium, du sodium et du chlore chez l'homme normal. Annales Médecine, *54*, 431–454 (1953).
6. *Ghata, J.*, and *Reinberg, A.:* Variations nycthémérale, saisonnière et géographique de l'élimination urinaire du potassium et de l'eau chez l'homme adulte sain. Comptes Rendus Ac. Sc. (Paris), *239*, 1680–1682 (1954).
7. *Reinberg, A.*, and *Ghata, J.:* Les rythmes biologiques. Presses Univ. France. Paris, 128 pp (2nd Edition), 1964.
8. *Reinberg, A.:* Le potassium et la vie. Presses Univ. France. Paris 128 pp. (2nd Edition), 1969.
9. *Kleitman, N.:* Sleep and wakefulness (Univ. Chicago 552 pp.), 1965.
10. *Mills, J.N.:* Human circadian rhythms. Physiol. Rev. *46*, 128–171 (1966).
11. *Kayser, C.*, and *Heusner, A.A.:* Le rythme nycthéméral de la dépense d'énergie. Etude de Physiologie comparée. J. Physiol. (Paris), *59*, 3–117 (1967).
12. *Halberg, F.:* Temporal coordination of physiologic function. In: Cold Spring Harbor Symp. Quant. Biol., New York. Long Island, Biol. Ass. N.Y. *25;* 289–310 (1960).
13. *Halberg, F.:* Physiologic 24-hour rhythms. A determinant of response to environmental agents. In: Schaefer, K.E. Ed., Man's Dependance on the Earthly Atmosphere. MacMillan. N.Y. 48–69, (1962).
14. *Halberg, F.:* Circadian (about 24-hour) rhythms in experimental medicine. Proc. Roy. Soc. Med. *56*, 253–256 (1963).
15. *Halberg, F.:* Organisms as circadian systems: temporal analysis of their physiologic and pathologic responses, including injury and death. In Walter Reed Army Inst. of Res. Symp. on Medical Aspects of Stress in the Military Climate. Washington, D.C. 1–36, (1965).
16. *Reinberg, A.:* Hours of changing responsiveness in relation to allergy and the circadian adrenal cycle. In: Circadian Clocks. Aschoff, J. Ed. North-Holland Publ. Co., Amsterdam, 214–218, (1965).
17. *Halberg, F.*, and *Reinberg, A.:* Rythmes circadiens et rythmes de basses fréquences en physiologie humaine. J. Physiol. (Paris), *59*, 117–200 (1967).
18. *Reinberg, A.:* The hours of changing responsiveness or susceptibility. Perspectives Biol. Med. *11*, 111–128 (1967).
19. *Halberg, F.:* Chronobiology. Ann. Rev. Physiol. *31*, 675–725 (1969).
20. *Hellbrügge, Th.:* The development of circadian rhythms in infants. Biological clocks. Cold Spring Harbor Symp. Quant. Biol. Long Island. Biol. Assoc. *25*, 311–323 (1960).
21 *Hellbrügge, Th., Ehrengut Lange, J., Rutenfranz, J.*, and *Stehr, K.:* Circadian periodicity of physiological functions in different stages of infency and childhood. Ann. New York Acad. Science, *117*, (art. 1), 361–373 (1964).
22. *Reinberg, A.:* Evaluation of circadian dyschronism during transmeridian flights. Studium Generale, *23*, 1159–1168 (1970).
23. *Azerad, E., Ghata, J.*, and *Reinberg, A.:* Disparition du rythme nycthéméral de la diurèse et de la kaliurie dans 8 cas d'insuffisance surrénale. Ann. Endocrinol. Paris, *18*, 484–491 (1957).
24. *Ghata, J.:* Etude des fonctions rénales, réabsorption de l'eau et des électrolytes et rythme nythéméral des éliminations chez l'addisonien traité par la cortisone. Sem. Hôp. Paris (Path. et Biol.) *32*, 84–89 (1956).
25. *Bartter, F.C., Delea, C.S.*, and *Halberg, F.:* A map of blood and urinary changes related to circadian variations in adrenal cortical function in normal subjects. Ann. N.Y. Acad. Sc., *98*, (4), 969–983 (1962).
26. *Reinberg, A., Ghata, J., Halberg, F., Apfelbaum, M., Gervais, P., Boudon, P., Abulker, Ch.*, and *Dupont, J.:* Distribution temporelle du traitement de l'insuffisance corticosurrénalienne. Essai de chronothérapeutique. Ann. Endocrinol. Paris *32* (4), 566–573 (1971).

27. *Halberg, F.:* Ritmos y corteza suprarenal. IV — Simposio panamericano de farmacologia y terapeutica Mexico, 1967 — Excerpta Medica Int. Cong. Ser., *185*, 7–39 (1967).
28. *Halberg, F., Bartter, F.C., Nelson, W., Doe, R.,* and *Reinberg, A.:* Chronobiologie. J. Europ. Toxicol. *2* (N° 6), 311–318 (1969).
29. *Halberg, F.,* and *Panofsky, H.:* Thermo-variance spectra; method and clinical illustration. Exp. Med. Surg. *19*, 284–309 (1961).
30. *Halberg, F, Panofsky, H., Diffley, M., Stein, M.,* and *Adkins, G.:* Computer techniques in the study of biologic rhythms. Ann. N.Y. Acad. Sci. *115*, 695–720 (1964).
31. *Halberg, F., Engeli, M., Hamburger, C.,* and *Hillman, D.:* Spectral resolution of low frequency, smallamplitude rhythms in excreted ketosteroid. Acta Endoc. Suppl., *103*, 54 pp (1965).
32. *Halberg, F., Tong, Y.L.,* and *Johnson, E.A.:* Circadian system phase: an aspect of temporal morphology procedure and illustrative examples, in: the Cellular Aspects of Biorhythms. Symp. on rhythmic Research, 8th Int. Cong. Anat. Springer Verlag, Berlin, 20–48, (1967).
33. *Halberg, F.:* Resolving power of electronic computers in chronopathology — an analogy to microscopy. Scientia, *101*, 412–419 (1966).
34. *Halberg, F.:* Physiologic 24-hour periodicity: general and procedural considerations with reference to the adrenal cycle. Z. f. Vitamin-Hormon- und Fermentforschung, *10*, 225–296 (1959).
35. *Reinberg, A.:* La chronobiologie: une étape nouvelle de l'étude des rythmes biologiques. Sciences, *1*, (no. 4), 181–197 (1970).
36. *Reinberg, A.:* Biorythmes et chronobiolgie — Presse Méd., *77*, 877–878 (1969).
37. *Reinberg, A.:* L'homme et les rythmes circadiens. Cahiers Sandoz, *8*, Paris, 50 pp (1966).
38. *Bünning, E.:* The Physiological Clock. (Springer Verlag, Berlin, 145 pp, (1964).
39. *Menzel, W.:* Menschliche Tag-Nacht-Rhythmik und Schichtarbeit. Benno Schwabe, Basel/Stuttgart, 189 pp, (1962).
40. *Sollberger, A.:* Biological Rhythm Research. Elsevier, New York, 461 pp, (1965).
41. *Cloudsley-Thompson, J.L.:* Rhythmic Activity in animal Physiology and Behavior, Academic Press, New York, 236 pp, (1961).
42. *Harker, J.E.:* The Physiology of Diurnal Rhythms Cambridge Univ. Press, London, 114 pp, (1964).
43. *Circadian Clocks:* Proc. Feldafing Summer School Aschoff, J. Ed. North-Holland, Amsterdam, 479 pp.
44. *Biological Clocks:* Cold Spring Harbor Symp. Quant. Biol. Long Island Biol. Assoc., New York, 524 pp., (1960).
45. *Rhythmic Functions in the Living System:* Ann. N.Y. Acad. Sci. Wolf, W. Ed. New York, 1326 pp, (1962).
46. *Circadian Systems:* Rept. 39th Ross Conf. Pediat. Res. Fomon, S.F. Ed. Ross Labs. Columbus, Ohio, 93 p, (1961)
47. *Photo-Neuro-Endocrine Effects in Circadian Systems with Particular Reference to the Eye:* Ann. N.Y. Acad. Sci. Hague, E.B. Ed. New York, 645 pp, (1964).
48. *Symposium on Rhythms:* In Verhandl. Verhandl. Ges. Inn. Med. 33. Kongr. 886–994, 1116–1117. Bergmann, München, 1967.
49. *Proc. 1st Intern. Symp. Biorhythms in Exptl. Clin. Endocrinol:* Florence, May 30–31, Rass. Neurol. Veget; 1966.
50. *La photorégulation de la reproduction chez les oiseaux et les Mammifères:* Colloq. Inter. C.N.R.S. Montpellier 1967. *Benoît, J., Assenmacher, I.,* Eds. CNRS, Paris, (no. 172) 588 pp, (1970).
51. *Biological Cycles* and *Psychiatry:* 3ème Symp. Bel-Air Genève, *J. de Ajuriaguerra,* Ed. Masson Publ. Paris, 422 pp, (1968).
52. *Aschoff, J.:* Comparative physiology; diurnal rhythms. Ann. Rev. Physiol. *25*, 581–600 (1963).
53. *Vanden Driessche, Th.:* The role of the nucleus in the circadian rhythms of Acetabularia mediterranea. Biochim. Biophys. Acta, *126*, 456–470 (1966).
54. *Pittendrigh, C.S.:* On Temporal organization in living systems. The Harvey Lectures, *56*, 93–125 (1961).
55. *Apfelbaum, M., Reinberg, A., Nillus, P.,* and *Halberg, F.:* Rythmes circadiens de l'alternance veille-sommeil pendant l'isolement souterrain de sept jeunes femmes. Presse Méd., *77*, 879–882 (1969).
56. *Reinberg, A., Halberg, F., Ghata, J.,* and *Siffre, M.:* Spectre thermique (rythmes de la température rectale) d'une femme adulte saine avant, pendant et après son isolement souterrain de trois mois. Comptes Rendus Ac. Sci. (Paris), *262*, 782–785 (1966).
57. *Ghata, J., Halberg, F., Reinberg, A.,* and *Siffre, M.:* Rythmes circadiens désynchronisés du cycle social (17-hydroxycorticostéroides, température rectale, veille-sommeil) chez deux adultes sains. Ann. Endocrinol. (Paris), *30*, 245–260 (1969).
58. *Reinberg, A.:* Methodologic considerations for human chronobiology. J. Interdisc. Cycle Research *2*, 1–15 (1971).
59. *Halberg, F., Visscher, M.B.,* and *Bittner, J.J.:* Relation of visual factors to eosinophil rhythm in mice. Amer. J. Physiol. *179*, 229–235 (1954).
60. *Aschoff, J.:* Zeitgeber der tierischen Tagesperiodik. Naturwissenschaften, *41*, 49–56 (1954).
61. *Pittendrigh, C.S.,* and *Bruce, V.G.:* An oscillator model for biological clocks. In: Rhythmic and synthetic processes in growth. Princeton Univ. Press. Princeton N.J., 75–109, (1957).
62. *Hoffmann, K.:* Die relative Wirksamkeit von Zeitgebern. Oecologia (Berlin) *3*, 184–206 (1969).
63. *Haus, E.,* and *Halberg, F.:* Circadian phase diagrams of oral temperature and urinary functions in a healthy man studied 'longitudinally'. Acta Endocrinol. *51*, 215–223 (1966).
64. *Halberg, F., Reinhardt, J., Bartter, F.C., Delea, C., Gordon, R., Reinberg, A., Ghata, J., Halhuber, M., Hofman, H., Gunther, R., Knapp, E., Pena, J.C.,* and *Garcia-Sainz, M.:* Agreement in endpoints from

circadian rhythmometry on healthy human beings living on different continents. Experientia, *25*, 107 (1969).
65. *Reinberg, A., Ghata, J., Halberg, F., Gervais, P., Abulker, Ch., Dupont, J.*, and *Gaudeau, Cl.:* Rythmes circadiens du pouls, de la pression artérielle, des excrétions urinaires en 17-hydroxycorticostéroides, catécholamines et potassium chez l'Homme adulte sain, actif et au repos. Ann. Endocrinol. (Paris), *31*, 277–287 (1970).
66. *Wesson, K.G.:* Electrolyte excretion in relation to diurnal cycles of renal functions. Medicine, *43*, 547–592 (1964).
67. *Stamm, D.:* Tagschwankungeb der Normalbereiche diagnostisch wichtiger Blutbestandteile. Werh. Dtsch. Gesell. Inn. Med. *73*, 982–989 (1967).
68. *Halberg, F.:* Grundlagenforschung zur Ätiologie des Karzinoms. Monatskurse f. d. ärztliche Fortbildung. *14*, 67–77 (1964).
69. *Mills, J.N.*, and *Stanbury, S.W.:* A reciprocal relationships between K^+ and H^+ excretion in the diurnal excretory rhythm in man. Clin. Sci., *13*, 177–186 (1954).
70. *Mills, J.N.*, and *Stanbury, S.W.:* Rhythmic diurnal variations in the behavior of the human tubule. Acta Med. Scand. Suppl. *307*, 95–96 (1955).
71. *Mills, J.N.*, and *Stanbury, S.W.:* Persistant 24-hour renal excretory rhythm on a 12-hour cycle of activity. J. Physiol. (London), *117*, 22–37 (1952).
72. *Imrie, M.J., Mills, J.N.*, and *Willamson, K.S.:* Circadian variations in renal and adrenal functions are they connected? Hormones and the kidney. Mem. Soc. Endocrinol. (Academic Press), *13*, 3–13 (1969).
73. *Siffre, M., Reinberg, A., Halberg, F., Ghata, J., Perdriel, G.*, and *Slind, R.:* L'isolement souterrain prolongé. Etude de deux sujets adultes sains avant, pendant et après cet isolement. Presse Médicale, *74*, 915–919 (1966).
74. *Halberg, F., Nelson, W., Doe, R., Bartter, F.*, and *Reinberg, A.:* Chornobiologie. J. Europ. Toxicol., *2*, (6), 311–318 (1969).
75. *Conroy, R.T.W.L.*, and *Mills, J.N.:* Human circadian rhythms. J. & A. Churcill. London, 236 pp, 1970.
76. *Reinberg, A., Zagulla-Mally, Z., Ghata, J.*, and *Halberg, F.:* Circadian reactivity rhythm of human skin to house dust, penicillin and histamine. J. Allergy, *44*, 292–306 (1969).
77. *Klein, K.E., Wegmann, H.M.*, and *Brüner, H.:* Circadian rhythm in indices of human performance, physical fitness and stress resistance. Aerospace Med. *39*, 512–518 (1968).
78. *Lobban, M.C.:* Human renal diurnal rhythms in an Arctic mining community. J. Physiol. (London), *165*, 75–76 (1963).
79. *Simpson, H.W.:* Studies on the daily rhythm of the adrenal cortex. Ph. d. Thesis. University of Glasgow, 1965.
80. *Simpson, H.W.*, and *Lobban, M.C.:* Effect of a 21-hour day on the human circadian excretory rhythms of 17-hydroxycorticosteroids and electrolytes. Aero. Med., *38*, 1205–1213 (1967).
81. *Simpson, H.W., Lobban, M.C.*, and *Halberg, F.:* Arctic chronobiology. Urinary near 24-hour rhythms in subjects living on a 21-hour routine in the arctic. Arctic anthropology. VII-I, 144–164 (1970).
82. *Haus, E.*, and *Halberg, F.:* Circannual rhythm in level and timing of serum corticosterone in standardized inbred mature mice. Environmental Research, *3*, 81–106 (1970).
83. *Board, F., Wadeson, R.*, and *Persky, H.:* Depressive affect and endocrine functions. Arch. Neurol. Psychiatr., Chicago, *78*, 612–620 (1957).
84. *Goldman, R.:* Studies in diurnal variation of water and electrolyte excretion: nocturnal diuresis of water and sodium in congestive cardiac failure and cirrhosis of the liver. J. Clin. Invest. *30*, 1191–1199 (1951).
85. *Liddle, G.W.:* Analysis of circadian rhythms in human adrenocortical secretory activity. Arch. Intern. Med. *117*, 739–743 (1966).
86. *Reinberg, A.:* Rythmes des fonctions corticosurrénaliennes et systèmes circadiens. In: Problèmes actuels d'Endocrinologie et de Nutrition. Vol. *10*, H.P. Klotz et J. Tremolières Ed. Expansion. Paris. pp. 75–90, (1966).
87. *Halberg, F., Visscher, M.B., Flink, E.B., Berge, K.*, and *Bock, F.:* Diurnal rhythmic changes in blood eosinophil levels in health and in certain diseases. J. Lancet, *71*, 312–319 (1951).
88. *Halberg, F.:* Methodological aspects of studies on physiological rhythms with special reference to the adrenal cycle. 5th Conf. Soc. biol. Rhythm. Stockholm. 1955, Rep. 98 (1961).
89. *Doe, R.:* A study of the circadian variation in adrenal function and related rhythms. Ph. D. Thesis. University of Minnesota. June 1966, (1966).
90. *Reinberg, A.*, and *Halberg, F.:* Circadian chronopharmacology. Ann. Review. Pharmacol. (1971).
91. *Lunedei, A., Cagnoni, M., Fantini, F., Tarquini, B., Morace, G., Maiello, M., Panerai, A. Scarpelli, P.T.*, and *Toccafondi, R.:* Sindromi Diencefaliche (Problemi in discussione), L. Pozzi, Roma, 413 pp., (1967).
92. *Reindl, K., Falliers, C., Halberg, F., Chai, H., Hillman, D.*, and *Nelson, W.:* Circadian acrophases in peak expiratory flow rate and urinary electrolyte excretion of asthmatic children: phase shifting of rhythms by prednisone given in different circadian system phases. Rass. Neurol. Veg. *23*, 5–26 (1969).
93. *Halberg, F.:* Ritmos y corteza suprarrenal. IV – Simposio panamericano de farmacologia y terapeutica. Mexico. Excerpta Medica Internat. Congr. no. 185, 7–39 (1967).
94. *Reinberg, A, Zagulla-Mally, Z., Ghata, J.*, and *Halberg, F.:* Circadian rhythm in duration of salicylate excretion referred to phase of excretory rhythms and routine; Proc. Soc. Expr. Biol. Med., *124*, 826–832 (1967).
95. *Aschoff, J.:* Eigenschaften der menschlichen Tagesperiodik. Arbeitsmedizin, Sozialmedizin, Arbeitshygiene *38*, 21–43, A.W. Gentner Verlag, Stuttgart (1970).

The Therapeutic Use of Potassium Salts

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Potassium plays an important and increasing role in therapeutics. This is explained by the fact that a lot of active drugs have the side effect of inducing a loss of K. Therapeutics have thus become the principal cause of hypokaliemia, coming before pathological causes.

This paper is made of three parts:

1. Treatment liable to induce potassium deficiency.
2. Correction of potassium deficiency.
3. Indications of potassium salt therapy.

1. *Treatments liable to induce potassium deficiency*

Certain medical and surgical therapies are liable to induce potassium deficiency.

1.1 *Diuretics*

Physiologically, potassium filtered by the glomerulus is, on one hand, reabsorbed completely by the proximal convoluted tubule and, on the other hand, secreted in more or less abundant quantity by the distal tubule.

All the diuretics, except the aldosterone antagonists, are liable to induce a potassium deficiency.

The action of various diuretics differs. Without going into the action on the renal vascularization and the permeability of the nephron, they can be divided into two groups: those that act upon the reabsorption of sodium throughout the nephron (thiazides, furosemide, ethacrynic acid); those that interfere with the exchange mechanism between sodium and potassium at the distal tubule level (triamterene).

There are two mechanisms for active transport of sodium: in the first case, the phenomena is independent of the secretion of potassium; in the second case, the sodium-transporting mechanisms are dependent on the secretion of potassium at the level of the distal tubule.

1.2 *The diuretics inducing a high degree of K elimination* (Table 1)

The thiazides, furosemide and ethacrynic acid all belong to the group which act throughout the nephron on the reabsorption of sodium. They also block potassium reabsorption. They have a protracted action and an increased efficacity if the urine is acid.

Chlortalidone, and the thiazides have also an inhibiting action on the carbonic anhydrase. Ethacrynic acid, which does not have this kind of actions, is even more active; it may act when the other diuretics have lost their efficiency, so is furosemide.

All these diuretics induce at the distal tubule level an increased potassium secretion and a sodium retention which is, however, not adequate to cancel out the effects at the proximal level; the potassium deficiency is accompanied by a loss of chloride. The consequence is that all these diuretics are likely to set up a metabolic hypochloremic hypokalemic alkalosis. The urinary potassium excretion in the 3 days following the administration of a diuretic varies between 190 and 300 meq, i.e. approximately of 12 g. This extension of the K deficiency is largely independent of the elimination of sodium and it persists and even increases while the effects of the sodium loss in the urine are not very marked. When the diuresis does not show a clear increase, it is dangerous to continue with the administration of diuretics. Practically speaking, *chlorothiazide and its derivatives* cause a high degree of depletion which is often very curt. Chlortalidone (Hygroton) has a more protracted and less brutal action on the hydro-sodic diuresis, the potassium depletion would be less here than in the case of chlorothiazide.

Furosemide (Lasilix) causes a smaller K loss and is more efficient than those aforementioned in bringing about diuresis; its action is less protracted.

Ethacrynic acid is the most active of all the diuretics while the potassium loss remains moderate within the limits of the usual dose.

Acetazolamide acts only as an inhibitor of the carbonic anhydrase, inducing a loss of alkaline radicals in the form of bicarbonates, in other words a metabolic acidosis; it induces a marked potassium depletion.

Table 1.

Diuretics	*K^+ urinary elimination*
Acetazolamide	+ + +
Chlorothiazide and its derivatives	+ + +
Chlortalidone	+ +
Furosemide	+
Ethacrynic acid	+

1.2 *Corticoids* (Table 2)

All the corticoids can cause hypokaliemia and require a systematic supply of K. This varies according to the type of corticoid and its posology.

Table 2.

Corticoids	*Loss of potassium*
Cortisone	+ + +
Prednisone	+ +
Methylprednisolone	+
Triamicinolone	+
Dexamethasone	+ +

It is admitted that the loss of the K ion represents in weight about twenty times the dose of prednisone administered (i.e. approximately 100 mg of K^+ per 5 mg of prednisone). Certain preparations combine corticoid and K-salt in the same application. This method

has the advantage of reducing the number of tablets to be absorbed, but it lacks flexibility and does not always allow a correction of the potassium dosage and its adaptation to the case in question. In practice, for a daily dose equal to or less than 15 mg of prednisone, a diet rich in K will be sufficient to compensate the losses which in this case are moderate. For a higher dosage, it will be necessary to add a supply.
Moreover, cortisone, hydrocortisone and acetate of desoxycorticosterone all involve a marked K loss, but these hormones – which are no longer used except as replacement in the course of adrenal insufficiency – are intended to correct preexisting electrolytic disturbances. It would therefore be illogical to give supplementary K.

1.3 *Liquorice extract*

Liquorice extract, whose main active element is glycyrrhizic acid, has mineralocorticoid effects. Its protracted administration in high doses (for purposes of sweetening the P.A.S. or the drinking water used during alcoholics detoxication cures) can bring about K losses of such importance as to necessitate the prescription of K salts.

1.4 *Laxatives*

The potassium depletion occurring during the excessive and long-duration use of laxatives is a well-known phenomenon. The effect of certain medications such as phenolphthalein is particularly dangerous: irritation of the intestinal mucosae leads to hypersecretion and to increased ionic elimination.
The frequent psychopathic context of these patients sometimes makes it difficult to track down the etiology of this hypokaliemia. It is frequently a question of phobias connected with constipation, or obesity, which try to hide the routine ingestion of laxatives. It must be remembered that only digestive losses can induce hypokaliemia with hypokaliuria.
The global K deficiency is not necessarily parallel to the level of the kaliemia because it can be masked by the metabolic acidosis resulting from the diarrhoea.
The corrective treatment includes two points:
- Suppression of the responsible laxative, constipation should be treated by means of essentially hygienic and dietetic measures;
- K deficiency is sometimes important enough to necessitate the medicamentous prescription. The dosage, however, should be done with great prudence, taking care not to exceed a daily dose of 4–6 g because a rapid and massive additional supply could induce the appearance of edemas. Any gain in weight would encourage the patient to start taking laxatives again without medical prescription.

1.5 *During diabetic acidoketosis treatment*

In the course of treatment, diabetic acidoketosis may be complicated by a serious hypokaliemia. This is explained by various reasons: under the effect of insulin, the potassium necessary for the glycogenesis penetrates in massive quantities into the cells hence the hypokaliemia which is further aggravated by the re-expansion of the extra-

cellular space caused by rehydration by liquids lacking in K and made even worse by the renewal of the diuresis.
This syndrome, which is secondary to the therapy, will appear towards the fifth hour of treatment.
The hyperosmolar coma brings with it osmotic diuresis because of the enormous degree of hyperglycemia. Kaliuresis is high. Massive additional supplies of K (14 g per day) therefore become necessary, perfused with hypotonic aqueous solutions. Its administration in the form of chloride should be avoided as it is not easily eliminated by this dehydrated organism and there is the risk of causing hyperchloremic acidosis.

1.6 *Other important problems of reanimation and the hydro-electrolytic re-equilibration may be encountered:*

a) *During barbituric coma treatment* by administration of large quantities of bicarbonates, the metabolic alkalosis sought for the facilitation of the purge increases the tubular excretion of K^+ ions. Artificial respiration also induces hypokaliemia. It is also necessary to add to each litre of bicarbonic aqueous solution 3—4 g of ClK. In the case of hyperchloremic acidosis patients, potassium bicarbonate should be used.
b) *In the case of neuro-respiratory reanimation,* hypokaliemia is due to the correction of the asphyxia and the gaseous acidosis by artificial respiration. Many other factors can also be present: rehydration, administration of diuretics, corticoids and bicarbonates.
c) *Extra-renal purification* by peritoneal dialysis or artificial kidney can also induce hypokaliemia; frequent biological checks are necessary.
Those cases of hypokaliemia should also be mentioned here that occur upon resumption of diuresis in the course of acute anuric nephritis. As the K loss can reach 200—300 meq/24 h, a massive ionic supplement will be necessary.
Finally, in the course of chronic nephritis, the strict lipidoglucidic diet, vomiting and diarrhoea — so frequent in these cases — contribute to explain the occurrence of hypokaliemia.

1.7 *Cation exchange resins*

The cation exchange resins are used to correct hyperkaliemia because they bring with them K intestinal elimination. The type is that of sulfonate of sodio-polystirene (Kayexalate®) which fixes 1—2 meq of K per gramme of resin. The kaliemia diminishes approximately of 1 meq in 12 hours. Here, no additional supply of potassium is justified because it is a question of normalizing an already high kaliemia.

1.8 *Surgical therapeutics*

a) *Systematic studies of K balances* have proved that every surgical intervention is followed by an increased kaliuresis. This fact, neglected earlier on, becomes interesting if other causes of hypokaliemia are also present: Fasting, increased extra-renal losses by vomiting, fistulas or continued aspirations, because all the digestive fluids are rich in K (Table 3).

Table 3.

	Concentration of K (mg/l)	Quantity secreted (l/24 h)	Quantity of K secreted (mg/24 h)
Saliva	320 to 1200	1.5	900
Gastric juice	400 to 800	2.5	1000 to 2000
Bile	200	0.5	100
Pancreatic juice	200	0.7	140
Normal excrements	3900 to 7800	0.5	2000 to 4000

b) *Continuous gastro-duodenal aspirations* are well tolerated if they are of short duration. If, however, they are protracted, it must not be forgotten that the gastric liquid contains 10—30 meq/l of K^+, that no food is being given and that the aspiration may have been preceded by vomiting.
If the additional administration of K salts is insufficient, hypokaliemia will appear and can transform itself into a subocclusive state necessitating the maintenance of aspiration. The treatment must include the administration of potassium salts and the re-establishment of chloride status.
c) *Biliary, pancreatic and intestinal fistulas* can induce a severe potassium depletion. In fact, the loss of these alkaline secretions lead to hypokaliemia with hyperchloremic acidosis. This acidosis helps to mask the importance of the actual shortage of potassium.
d) The same applies in the case of *ureterosigmoidostomy* (*Coffey's* operation). Hypokaliemia following ureterocolic anastomosis is accompanied by hyperchloremic acidosis.
e) The surgical amputation of adrenocortical tumours responsible for hyperaldosteronism (*Conn* syndrome) demands ionic control and preoperative correction of hypokaliemia.
f) In the case of *open heart surgery*, exchangeable potassium measurements have shown a value below the normal or at the lower limit of the normal. When extra-corporal circulation is taking place, kaliemia can be very low in spite of the addition of potassium salts to the circuit. During the post-operative phase, the main cause of potassium depletion is the high degree of diuresis. Any depletion must be corrected, particularly in the case of patients to whom digitalin is administered.

2. *Correction of potassium deficiency*

Hypokaliemia is only a symptom, the treatment of which is the suppression of its cause. In the meantime, K deficiency being in itself the cause of severe troubles, it frequently becomes necessary to correct hypokaliemia as quickly as possible.
The correction of K deficiencies is possible in two ways: a suitable diet and medication.

2.1 *Diet*

By a judiciously balanced quantitative and qualitative choice of foods, an increased supply of K can be administered. A normally balanced diet supplies 3 to 4 g of K ion per day. This quantity is sometimes insufficient and necessitates the addition to the diet of foods rich in K such as fruit juice (1,50—1.90 g/l), carrot juice (0.60 g/l), cow's milk (0.45 g/l)...

But the possibilities of a diet such as this are limited because of the necessity of weighing the foods, because of the risk of inflicting upon the patient a diet of a unacceptable monotony for more than a short time. Moreover, even if one succeeded in imposing such a strict diet, the quantities of K taken might still be insufficient in case of very considerable depletion, such as can be suddenly caused by certain therapies (Table 4).

Table 4. Classification of foods according to their K content

Foods and beverage	Foods very rich in K (> 700 mg/100 g)	Foods rich in K (400–700 mg/100 g)	Foods with a moderate K content (100–400 mg/100 g)	Foods poor in K (< 100 mg/100 g)
Fruit and nuts	Almonds Dates Dried figs Dried prunes Dried grapes Dried apricots	Apricots Bananas Nuts	Other fresh fruits	
Vegetables	Dried vegetables (lentils, haricot, beans) Soya Spinach	Potatoes Beetroot Carrots Mushrooms	Other fresh vegetables	
Dairy produce Eggs			Whole milk Yoghourt Fermented cheese Eggs	Fresh cheese
Meat Fish		Sardines in oil	Meat Poultry Fish Shrimps Oysters	
Cereals		Wholemeal flour	Italian paste White flour Wholemeal bread Rusks	Tapioca Rice Semolina White bread
Fats				Butter Oil Other fats
Sugar products	Cocoa Molasses + + +	Chocolate		Sugar Jam Honey
Beverages			Fruit juice	Beer Cider Wine 12°

2.2 *Medication*

In order to restore to the body a 'working capital' of potassium, it must be remembered that:

- all the K administered is not retained,
- the method of administration depends on the etiology,
- it must always be done in conjuction with rehydration,
- sodium, although useful when given in small doses, becomes toxic when administered in larger doses.

A patient suffering from K deficiency can be 'restocked' either orally or parenterally.

2.2.1 *Oral administration*

The use of K salts has the advantage of providing a quantity of electrolyte easily measurable; the salts most commonly used are: chloride, phosphate, citrate. Chloride contains about half its weight in K, lactate contains only a third (Table 5).

Table 5. Composition of potassium salts used in therapeutics

Formula	Molecular weight	Percentage of K	Quantity providing 1 g of K
Chloride	75	52	1.92
Citrate	306	12.8	7.81
Lactate	128	30.5	3.28
Gluconate	234	16.7	5.99
Propionate	112	34.8	2.87

These salts, administered in fractionated doses of 2 g up to a total of 10 g per 24 hours, may be added to a sweetening syrup (lemon, orange) or to an unsalted meat broth, in order to make the salts less unpleasant.
Vomiting, diarrhoea and continuous aspirations sometimes make the oral administration of these salts ineffective. Injectable aqueous solutions (or a *Murphy* drip) will then become necessary.

2.2.2 *Parenteral administration*

Chloride and phosphate are K salts most commonly used. *Chloride* is ideal because a deficiency of chloride is often associated with K deficiency, as we have already seen. *Phosphate* is a salt whose use is also logical because phosphate is an intracellular anion, a deficiency of which frequently appears in conjuction with K deficiency. Monopotassic and dipotassic phosphates can be associated so that the pH is maintained at 7.35. These K salts are prepared in the form of hypertonic solutions to be diluted before use.

Potassium salts suitable for parenteral administration
(Hypertonic solutions to be diluted before use)

- *Potassium chloride* at 10/100
  10 ml = 1 g KCl = 520 mg or 13 meq of K
  20 ml = 2 g KCl = 1040 mg or 26 meq of K
- *Potassium citrate* at 10/100
  10 ml = 1 g of citrate = 128 mg or 3.2 meq of K
- *Potassium propionate* at 14.5/100
  20 ml = 2.9 = 1000 mg or 25 meq of K
- *Potassium lactate* at 12.8/100
  10 ml = 1.28 g = 390 mg or 10 meq of K.

The easiest method of administration is to add a potassium salt to the principal isotonic aqueous solutions (salted or glucosed).

Numerous injectable aqueous solutions have been suggested. They must be stable, isotonic and suitable for intravenous injection. The oldest of these – *Ringer's* solution suggested in the treatment of diarrhoea in infants – may be used so long as all the following precautions are taken:
– the daily dose must not exceed 80 ml per kilogramme;
– the speed of injection should be such that the perfusion lasts between 8 and 12 hours;
– the perfusion should be undertaken only if renal function is normal.
The daily dose which can be administered intravenously is from 6 to 8 g (150 to 200 meq) of potassium in the case of an adult and between 2 and 4 meq/kg per 24 hours in the case of a child. Such quantities as these should always be administered slowly and in dilution. It is wise not to exceed 2–3 g of potassium per litre of aqueous solution and not to inject more than 1 g during the first hour, then 0.75 g in the following hours.
These aqueous solutions can also be administered by an intestinal or gastric tube, as a drip.
At all events, K salts must be administered until kaliemia become normalized and resumption of a normal diet is possible. The treatment should be observed by means of humoral and electrocardiographic controls in order that the correction of the kaliemia troubles may be carefully followed. An excessive correction, in other words the appearance of signs of hyperkaliemia, demands immediate cessation of the treatment.
Very considerable doses reaching and even exceeding 150 meq per day are sometimes necessary for the reconstitution of potassium capital.
1–2 g of calcium gluconate should be added if signs of tetany should appear in the course of the administration of potassium salts.

2.2.3 *Therapeutic accidents*

Attention should be drawn to the possible accidents which can occur during treatment with potassium salts.
For nearly 10 years, ulcerations of the small intestine (mainly on the initial part of the ileum) have been reported after absorption of potassium chloride tablets. This ulceration can be complicated by stenosis of the intestine, by perforations or haemorrhages. Affections such as these appear more readily after the absorption of large doses of potassium chloride but not only after prolonged treatment. The incidence of these accidents is, however, low.
If potassium chloride tablets are mainly involved, the use of aqueous solutions does not ensure total avoidance of such accidents. The other salts are not entirely safe; gluconate has been found responsible for similar affections. Tartrate, on the other hand, would be entirely innocuous, perhaps because of its lower content of potassium.
Potassium chloride maintains all its advantages so long as it is a question of a deficiency of chloride alone or associated with alkalosis. More recent preparations, being coated with a special substance, now appear to be less harmful for the small intestine than were the previous products.
Treatment with K salts intravenously also entails certain risks. The most frequent are the pain at the point of injection and venous sclerosis. The highest risk is that of a too rapid perfusion which can induce a sudden and transitory hyperkaliemia, because the plasmatic

potassium does not pass immediately to the interstitial and cellular sector. Here again the precautions already cited above must be observed.

2.2.4 *Conclusions*

The medication of patients afford a certain number of advantages:
- it can be effected under all circumstances orally or endovenously in the case of a patient in coma;
- it is quantitatively easily controllable;
- it can have a rapid action, quasi immediate when this is necessary;
- it can be adapted to various biological circumstances because it allows of the correction of associated ionic disturbances: a potassium deficiency with alkalosis – the most frequent eventuality – is treated by means of potassium chloride or citrate.

A potassium deficiency with hyperchloremic acidosis should be treated with one of the numerous K salts available to us: bicarbonate, lactate, tartrate, propionate, gluconate.

3. *Indications of potassium salts therapy*

3.1 *In nephrology*

The treatment with potassium salts is frequently inseparable from the use of diuretics. The administration of potassium must be adjusted according to the type of medication used, the duration of the treatment and the existence or non-existence of a renal insufficiency.
Apart from cases such as this, several particular affections need correction. The existence of *digestive losses* can add these hypokaliemic effects, independent of the urinary loss. Hyperaldosteronism, occurring secondarily to a urinary loss of sodium in the course of interstitial nephropathy, aggravated by an inopportune de-sodiumized diet, must be recognized and treated.
We mention a few chronic tubular insufficiencies congenital such as the *Fanconi* syndrome or hyperchloremic tubular acidosis; the K depletion in such cases would be linked with incapacity on the part of the kidney to produce acid urine. The diet should afford a large supply of potassium without containing too much glucide which could cause a brusque aggravation of hypokaliemia by intracellular captivation of K when sugar penetrates into the cell.
In practice, in the case of renal diseases, the choice of potassium salts would preferably fall upon non-alkalinizing potassium chloride. The rules concerning the total 24-hourly dose and the rate of intravenous perfusion already mentioned must be observed while taking into account the actual functioning of the kidneys. The potassium content may not exceed 100 meq/litre of liquid.
If a high degree of alkalosis is present, it is important that calcium salts are also given because of the risk of tetany at the moment when the potassium deficiency is corrected.
On the other hand, it must be remembered that the correction of a potassium depletion of some severity is sometimes followed by edemas which are perhaps the result of the transfer of sodium accumulated in the cells during the potassic depletion and which, on the arrival of fresh supplies of potassium in the body, return to the extracellular sector.

3.2 *In gastro-enterology*

The treatment with potassium salts is appropriate here as soon as there are sudden and extensive losses or moderate but protracted ones, either isolated but more frequently in association with other electrolytic disturbances.
The most immediate indication is the correction of potassium depletion, above all surgical and therapeutical (continuous gastro-duodenal aspiration, biliary, pancreatic and intestinal fistulas), but many other affections also require the administration of potassium salts.

3.2.1 *Prolonged gastro-intestinal affections*

Diarrhoea resulting from diffuse lesion of the colon and the small intestine can bring about considerable K losses. In the course of *Crohn's* disease, infectious jejunoileitis, malabsorption syndromes, *Zollinger-Ellison* syndrome, heavy diarrhoea with a high potassium content can bring about serious depletion in the long run.
The same applies in the case of hydro-electrolytic losses caused by *recto-sigmoidian villous tumours*, hypokaliemia accompanies hypochloremia and hyponatremia. In episodes of hemorrhagic rectocolitis, the fecal losses reach 25–30 meq/24 hours. The problem of the protracted addiction of laxatives, already mentioned, is a comparable case.
In all these conditions, it is important to point out that the loss of potassium in urine has a tendency to decrease, an important factor of orientation in favour of a digestive depletion.

3.2.2 *Acute digestive accidents*

In the course of *acute diarrhoea*, the replenishment of the body with potassium salts must take into account a loss of the order of between 50 and 150 meq/24 hours associated with extracellular dehydration of a considerable degree, with the added risk of metabolic acidosis.
In the course of *acute vomiting* (be it caused by intestinal occlusion, pyloric stenosis or an acute post-operative dilatation of the stomach), the replenishment will have to be very great. The supply can reach as much as 100 meq per hour; post-operative gastro-duodenal aspiration poses similar problems, as we have already seen.
In the course of decompensated *cirrhosis of the liver*, the treatment with potassium is the corollary to diuretic treatment, above all by means of the thiazides, not forgetting that frequent secondary hyperaldosteronism often necessitates recourse to the derivatives of spironolactone or triamterene either alone or in association with thiazides.

3.3.3 *In endocrinology*

In endocrinology, the treatment by potassium is indicated within the essential framework of adrenal cortex diseases.
In the course of treatment of diabetic coma, the importance of the administration of potassium at the moment of its ingress into the cell has already been mentioned.
In the case of *primary hyperaldosteronism*, metabolic alkalosis provoked by the adrenal

tumour is accompanied by a hypokaliemia that is generally moderate. The correction of this trouble should be undertaken before the surgical exeresis.
On the other hand, the incidence of *secondary hyperaldosteronism* caused by unilateral renal troubles, for example, is very frequent when the original disease has not beeen treated, thus causing hypokaliemia that subsequently requires treatment.
This potassium depletion can give rise to therapeutic problems of a special kind in the preparation for the intervention. It is in fact, responsible for operative complications such as disturbances of the cardiac rhythm, protracted respiratory paralysis, especially after use of curarines.
The correction of this potassium deficiency is essential and it is the task of the diet to achieve it: strict restriction of sodium, daily administration of 100 meq of supplementary potassium.
In the case of *Cushing's* syndrome, hypokaliemia generally remains moderate; potassium deficiency is greater in the course of paraneoplastic hypercorticisms. The problem is that of rapid surgical correction of the metabolic hyper-function.
All protracted cortico-therapy in large or moderate doses – already studied – demands a treatment in which the administration of potassium is included.
In case of *thyrotoxicosis*, the potassium pool, the exchangeable potassium, are diminished. This kaliopenia renders the patient suffering from thyrotoxicosis more sensitive to the additional potassic losses through diarrhoea or the inopportune administration of sali-diuretics. This explains, at least partially, the muscular syndrome, either isolated or associated with neurophysical disturbances.
The treatment of these complications demands hydro-electrolytic reanimation with, in particular, potassium supplementary to the specific treatment.

3.4 *In cardiology*

3.4.1 *Cardiac insufficiency*

The reasons for potassium depletion are numerous:
- *digestive troubles* (anorexia, vomiting) whether or not caused by the administration of digitalis;
- anoxia with loss of cellular potassium subsequently eliminated by the kidneys;
- catabolism, each gramme of nitrogen freeing 2.7 meq of potassium;
- finally, the protracted administration of diuretics can induce *secondary hyperaldo-steronism*.

Potassium depletion is essentially the result of a loss; it potentializes the digitalic effects, creating a risk of overdosage and intoxication.
The digitalics. Digitalin acts at the level of the cellular membrane by modifying the permeability of the electrolytes.
Subsequently, differences in the trans-membranes potential will become apparent. The consequences of this are, on the one hand, electric signs of digitalization and, on the other, a deceleration of the penetration of potassium into the cells. The effect of digitalin differs according to the dose administered, the initial state of the myocardium and of the intracellular potassium.
In toxic doses, digitalin lowers the potassium and the pH of the myocardium; the K

concentration in the coronary sinus increases in relation to that in the coronary arteries. There is a cellular loss of potassium and ingress of sodium. In cases of potassium depletion, digitalin can thus aggravate a cardiac insufficiency by hastening the loss of intracellular potassium.
For the correction of these losses, the administration of potassium is indispensable, but must be carried out with great prudence because the potassium penetrates less quickly into the cell in the case of extensive digitalization. The decrease in the glomerular filtration due to the cardiac insufficiency can cause a deceleration of the renal elimination; a too rapid increase of the kaliemia must be avoided. When correcting potassium deficiency, one must know of the risk of decreasing the cardiotonic activity inherent in the digitalics.

Treatment

The treatment is, above all, prophylactic. The observation of the kaliemia and the prescription of potassium salts administered *per os* must be systematic; the quantity to be given will depend on the degree of the kaliemia, the doses given being at least equal to the losses incurred. The curative treatment may necessitate intravenous administration. Doses are variable, about 100 meq/24 hours and given by slow perfusion so long as the renal elimination conditions are adequate and biologic and electrocardiographic control is exercised throughout.
In most cases, the administration of potassium in the diet will be sufficient, supplemented if necessary by dietetic salts without sodium. The supply afforded by the diet can be considered as adequate so long as there is no apparent sign of potassium depletion.
During rapid or extensive digitalization, additional potassium may prove necessary in the form of chloride if alkalosis is also present.
In the course of treatment with diuretics, supplementary potassium must be given as long as the diuretic effect lasts; this will depend on the diuretic being used. In the case of the sulfonamide diuretics, for example, an additional supply of between 2 and 3 g of potassium chloride per day, administered *per os,* is necessary if the kaliemia is within normal limits, and of between 5 and 10 g of KCl if there is hypokaliemia.
In addition to cases of cardiac insufficiency, potassium can be used in the treatment of digitalic intoxication, attacks of ventricular tachycardia of variable focus and also for myocardial infaction.

3.4.2 *Digitalic intoxication*

The potassium exercises a depressing effect on the excitability of the myocardium and thus has the opposite effect to that of digitalin. It has an inhibiting influence on the auriculo-ventricular conduction similar to that of digitalin. The administration of potassium salts is logically indicated when the digitalic intoxication manifests itself by myocardiac hyper-excitability: extra systoles or ventricular tachycardia. It is counter-indicated if there is an auriculo-ventricular block except in the case of a tachycardia auricular with auriculo-ventricular block. When the intoxication is light (bigeminal rhythm for example), oral administration is preferred. On the other hand, in the case of permanent hyper-excitability, the intravenous method must be chosen, the treatment being permanently observed both electrically and by ionogramme (the initial kaliemia can be marked, the salt

to be used is potassium chloride in slow perfusion, approximately 30 to 60 meq of K ion per 24 hours).

3.4.3 *Ventricular tachycardia*

In the cases of attacks of *ventricular tachycardia* of variable focus or torsade tip frequently induced by potassium depletion with metabolic alkalosis, the intravenous administration of potassium in conjunction with electro-systolic training (especially in the case of an auriculo-ventricular block) is regularly efficacious. On the other hand, in the case of disturbances of the cardiac rhythm (supraventricular tachycardia, bigeminal rhythm) which are caused neither by digitalic or quinidinic intoxication, nor again by potassic depletion, the administration of potassium is more questionable; it deserves, however, to be tried because of its relative innocuousness, so long as the rules of blood control by the ionogramme are observed and the perfusion is slow.

3.4.4 *Myocardial infarction*

In the case of *myocardial infarction,* potassium glucose insulin treatment is still a matter of controversy in as much as there are no positive criteria of its efficacity. However, it would seem that this therapy is able to shorten the duration of initial troubles of cardiac rhythm induced by infarction; in particular, fibrillation and ventricular tachycardia occur less frequently. Moreover, the pain-stilling becomes earlier and there is a more rapid resolution of the electro-cardiographic abnormalities.

3.5 *In neurology*

A very particular problem is posed by *Westphal's periodic paralysis.* The prophylactic treatment uses aldosterone antagonists (spironolactones).
Emergency treatment will consist in the intravenous administration of a solution of potassium chloride of 30–50 meq/l at the rate of 10 ml/kg/hour for two hours.

Conclusion

When prescribing treatment, the acido-basic balance of the patient should be taken into account.
Acidosis partly masks hypokaliemia; any decrease in the pH of 0.1 unit leads to an increase in the kaliemia of 0.6 meq/l. It counter-indicates the use of potassium chloride which is acidifying.
Alkalosis necessitates the prescription of potassium chloride; potassium salts of an organic acid (citrate, gluconate, tartrate) are alkalinizing and will not allow of correction of either alkalosis or hypokaliemia.

Bibliography

1. *Assan, R.:* Les accidents des diurétiques modernes, Rev. Prat. *33*, 4871 (1969).
2. *Azerad, E.*, and *Reinberg, F.:* Le métabolisme du potassium et ses troubles. Encyclopédie Méd. Chirurgicale (Nutrition) 10504, B10, Paris, 1965.
3. *Barlow, C.G.:* Release of potassium chloride from tablets. J. Pharm. Pharmacol. *17*, 822 (1965).
4. *Berger H.:* Potassium depletion in heart disease, in E. Bajusz: Electrolytes and cardiovascular diseases. Vol. 2, p. 17, Karger, Bâle, 1966.
5. *Bismuth, H., Samain, H.*, and *Martin, E.:* Les sténoses ulcéreuses du grêle après absorption de comprimés de potassium. La Press Méd. *74*, 1801–1804 (1966).
6. *Boley, S.J., Schultz, L., Schwartz, S., Katz, A.*, and *Allen, A.C.:* Potassium citrate and potassium gluconate versus potassium chloride. J.A.M.A. *199*, 215–218 (1967).
7. *Bonfils* and *Menburger, Ph.:* Indications diététiques du potassium en gastro-entérologie. Ann. Hyg. L. Fr. Méd. et Nut. *4*, 59–62 (1968).
8. *Bour, H.:* Les dyskaliémies. Rev. Prat. *15*, 3263–3717 (1965).
9. *Connoly, D.C.:* Arrhythmias associated with digitalis therapy. Postgrad Med. *25*, 509 (1959).
10. *Enrico, J.F., Pialino, M., Poli, S., et al:* Physiopathologie de l'alcalose métabolique. Schweiz. med. Wschr. *97*, 1587 (1967).
11. *Flear, C.T.*, and *Cawley, R.H.:* Potassium supplements and congestive heart failure. Brit. Heart J. *24*, 337 (1967).
12. *Fournier, A.:* La thérapeutique diurétique moderne. Bases physiologiques et physiopathologiques, Presse Méd. *78*, 705–716, 945–954 (1970).
13. *Goodman, J.R., Vincent, P.D.*, and *Rozen, R.:* Serum potassium changes in blood clots. Amer. J. Clin. Path. *24*, 111 (1954).
14. *Hazard, J.:* Le potassium dans l'organisme, répartition, métabolisme et régulation. Rev. du Prat. *15*, 3627–3637 (1965).
15. *Hazard, J.*, and *Duterque, M.:* Besoins potassiques de certaines thérapeutiques. Ann. Hyg. L. Fr. Méd. et Nut. *4*, 29–34 (1968).
16. *Kassirer, J.P., Berkman, P.M., Lawrenz, D.R., et al.:* The critical role of chloride in the correction of hypokaliemic alcalose in man. Amer. J. Med. *38*, 172 (1965).
17. *Leaf, A.*, and *Santos, R.F.:* Physiologic mechanisms in potassium deficiency. New-England J. Med. *264*, 335 (1961).
18. *Lestradet, H.:* Potassium et nutrition. Ann. Hyg. L. Fr. Méd. et Nut. *4*, 17–19 (1968).
19. *Lubetzki, J.:* Rations riches et pauvres en potassium. Ann. Hyg. L. Fr. Méd. et Nut. *4*, 21–27 (1968).
20. *Milliez, P.*, and *Lagrue, G.:* Potassium et pathologie rénale. Rev. du Prat. *15*, 3695–3702 (1965).
21. *Pick, A.:* Arrhythmias and potassium in man. Amer. Heart J. *72*, 295 (1966).
22. *Plattner, H.C.:* Le métabolisme du potassium. Masson, Paris, 1954, 1 vol., 155 pages.
23. *Reinberg, A.:* Potassium et santé humaine. Symp. 1966, p. 485–514, Institut International de la Potasse, Berne, 1966.
24. *Sadi Pallares, D., Bisteni, A., Medrano, G.A., et al.:* The polarizing treatment for myocardial infarction. Amer. J. Cardiol. *24*, 601 (1969).
25. *Taeschler, M.*, and *Perrottet, E.:* Conceptions actuelles sur le mécanisme d'action des glucosides cardiotoniques. Monographies Sandoz. Quelques notions actuelles sur les glucosides cardiotoniques. Sandoz, Basel, 1966.

The Role of Potassium in Nitrogen Balance – Experimental Evidence in Rat and Man

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We have studied, in the whole body and in several organs or tissues, the relationship between the potassium content and the protein-enzyme content.
The investigations were done on several groups of rats, differing in age and nutritional state.
The results, thus obtained, were compared with nitrogen and potassium balances in healthy human adults.

1. *Material and Methods*

1.1 *Animals*

Wistar male rats (Wistar Commentry) were subjected, since their weaning, to a semi-synthetic and adapted diet *(Apfelbaum [4])*.
Five groups of animals were considered: group I, II and III sacrified at the age of 6 months and group IV and V sacrified at the age of 18 months.
Group I ('young' control) 8 animals. Food available *ad libitum*. Body weight (b.w.), 313 ± 8.83 g at the age of 6 months (6mths).
Group II (acute starvation) 8 animals. Food available *ad libitum* excepted 7 days before killing. Hydric diet during these 7 days. 202 ± 13.10 g b.w. (6mths).
Group III (protein diet) 8 animals. Food available *ad libitum* excepted 7 days before killing. Water and 7 g of casein per day during these 7 days. 252 ± 4.70 g b.w. (6mths).
Group IV ('old' control) 20 animals. Food available *ad libitum*. 517 ± 16.3 g b.w. (18mths).
Group V (chronic undernutrition) 22 animals. After the age of two months, 50% intake reduction. 242 ± 3.47 g b.w. (18mths).
Exchangeable potassium determination
Rats were injected intra-peritoneally with ^{42}KCl. Specific activities were measured in urine *(Apfelbaum [3])*.
Fractionation
Animals were decapitated and bled (heparine) after 12 hours fasting. The following fractions were collected:
– liver
– quadriceps
– viscera (lungs, heart, spleen, kidneys, pancreas, stomach, intestine, etc.)
– skin (non defatted)
– the remaining fraction, called 'carcass'.

K^+ and N determinations

Each fraction was grinded, homogenized in water (except the skin, that was homogenized in 2*N* NaOH).

Potassium was extracted with trichloracetic acid. Total nitrogen was determined by the Kjeldahl method. Determination of soluble nitrogen – corresponding to the protein-enzyme nitrogen – was done after hydrolysis (0,05 *N* NaOH, 72 h – 37 °C) [*3, 4*].

The series of data, thus obtained, were submitted to statistical analysis and the results were expressed as means of concentration with their standard deviations ($\bar{x} \pm$ sd). The comparisons between ratios were done, after arc-sine transformation, and analysis of variance.

1.2 *Human subjects*

41 healthy obese women – from 14 to 56 years of age – weighing from 59.5 to 115.8 kg were hospitalized during a 21 days period. *(Boudon [12])* (Table 1).

Table 1. Morphological data

Initial body weight (b.w.)	90.87 kg
B.w. calculated from ideal weight/high ratio (Metrop. Life. Bull.) *[42, 43]*	59.42 kg
Estimated obesity (% b.w.)	45.37 kg
B.w. calculated from exchangeable K^+	52.59 kg
Estimated obesity (from exchangeable K^+) *(Apfelbaum [67])*	63.45 kg

Protein diet

The subjects received exclusively: 60 g of casein, 2 liters of water, vitamine and 1 or 3 g of potassium chloride *(Apfelbaum* [7]*)*.

Nitrogen and potassium balances were calculated every day according to a previously described method *(Apfelbaum* [*5, 8*]*)*.

Clinical and hormonal data have been already published *(Apfelbaum* [6]*)*.

1.3 *Rats*

Exchangeable potassium is equal to total potassium whatever the nutritional state (Table 2).

Table 2. Exchangeable and total potassium in the five experimental groups of rats, mEq/kg body weight

Groups	I: 'young' control	II: acute starvation	III: protein diet	IV: 'old' control	V: chronic undernutrition
Number of animals	8	8	8	20	22
Exchangeable K^+	57.2 ± 1.28	57.0 ± 1.88	61.7 ± 2.56	43.1 ± 2.08	56.2 ± 1.39
Chemical K^+	59.42 ± 0.197	57.8 ± 2.44	64.7 ± 1.02	43.8 ± 2.39	58.0 ± 2.03

Comparison between exchangeable and total potassium in the five experimental groups of rats (Table 3).

Table 3. K/soluble nitrogen ratios (meq/g)

Groups	I	II	III	IV	V
Number of animals	8	8	8	20	22
Whole animal	2.252 ±0.1820 NS	1.893 ±0.1485 NS	2.149 ±0.1722 NS	2.252 ±0.1183 *	2.459 ±0.1221 NS
Liver	2.537 ±0.1248 NS	2.543 ±0.2594 NS	2.360 ±0.2369 NS	2.816 ±0.1776 *	2.724 ±0.1569 NS
Quadriceps	2.928 ±0.2309 NS	3.064 ±0.2443 *	3.228 ±0.2788 *	3.315 ±0.1629 ***	3.070 ±0.1361 *
Viscera	3.389 ±0.3347 *	3.433 ±0.3402 ***	3.287 ±0.3220 *	2.898 ±0.1784 **	3.481 ±0.2253 **
Skin	1.154 ±0.1416 ***	0.918 ±0.1125 ***	0.988 ±0.1210 ***	1.012 ±0.0906 ***	0.985 ±0.0829 ***
Carcass	2.795 ±0.2390 NS	2.632 ±0.222 *	2.641 ±0.2229 *	2.906 ±0.1834 *	3.117 ±0.1828 *

NS: not significant difference $P > 0.05$ *: significant $P < 0.05$ **: significant $P < 0.01$ ***: significant $P < 0.001$

For experimental data see: *Apfelbaum [70] (a)*

Potassium/protein-enzyme nitrogen ratio in the five groups of rats [1].

1.4 *Human subjects*

The nitrogen balance is dependent on the potassium intake (Table 4).

Table 4. Nitrogen balance in man during the protein diet at two levels of potassium intake

Daily KCl intake	Age	No. of subjects	During 19 days	During the 1st week	During the 2nd week	During the 3rd week
1 g/d	under 25 years	4	0.17 ±0.388	−1.50 ±0.730	+0.64 ±0.564	+2.24 ±0.533
				$F = 6.18$ $P < 0.05$	$F = 3.48$ $P > 0.05$	
	over 25 years	8	−1.89 ±0.200	−3.41 ±0.364	−1.88 −0.232	−0.22 ±0.292
				$F = 12.46$ $P < 0.01$	$F = 19.69$ $P < 0.01$	
3 g/d	under 25 years	5	+3.52 ±0.239	+2.20 ±0.325	+3.81 ±0.234	+5.33 ±0.334
				$F = 16.77$ $P < 0.01$	$F = 10.83$ $P < 0.01$	
	over 25 years	4	−0.50 ±0.329	−2.21 ±0.553	−0.58 ±0.415	+1.38 ±0.544
				$F = 5.49$ $P < 0.05$	$F = 7.71$ $P < 0.01$	

For experimental data see: *Apfelbaum [70] (b)*

2. *Discussion*

2.1 *Exchangeable K^+ and total body K^+*

It has been demonstrated (Table 2) that in the five groups of rats the exchangeable K^+ is equal to total body potassium. Table 5 shows that this conclusion can also be drawn from data published by several investigators and reanalyzed from this point of view.

Table 5. Exchangeable and total potassium – Data from other authors

Authors	Year	Species	K_e meq/kg	Total K meq/kg
Harrisson and coll.	1936	dog	—	57.5
Noonan and coll.	1941	rat	76.9	66.5
		rabbit	43.5	72.9
Fenn and coll.	1941	frog	65.0	52.0
Levitt and Gaudinot	1949	dog	55.8	—
Corsa and coll.	1950	rabbit	56.1	58.6
Spray and Widdowson	1950	pig	—	70.0
Aikawa	1951	rabbit	62.0	—
Greene and Sapirstein	1952	rat	—	59.0
Ginsburg and Wilde	1954	rat	78.6	—
Cheek and West	1956	rat	—	65.4
Cier and coll.	1958	rat	—	64.6–65.0
Elkinton and coll.	1959	rat	—	65.4
Huth and Elkinton	1959	rat	—	73.3
Talso and coll.	1960	rat	71.0	72.7
Green and coll.	1961	pig	60.0	—
Lemley-Stone and coll.	1961	rat	58.0	—
Haxhe	1963	dog	53.7	—
		lamb	55.5	63.8
Kirton	1963	pig	50.0	—
Demanet	1964	rat	69.5	—

2.2 *Potassium and aging*

It can be seen on Table 2 that both total and exchangeable potassium concentrations, are lower in the 18 months rats than in the 6 months ones. These results are in good agreement with those, published by *Anderson* [2] (in man, using ^{40}K) by *Widdowson* [42] (in rat, photometric determinations) and by *Apfelbaum* [4] (in rat, using ^{42}K).

2.3 *The exchangeable K^+ is a good index of the cellular mass*

We have to keep in mind that the exchangeable potassium is not directly related to the lean body mass, but is related to the cellular mass. Even if differences in hydratation occur, and whatever the nutritional state and age, the potassium/protein-enzyme ratio remains constant.

Protein-enzyme (grammes) can be calculated from exchangeable potassium (meq) in multiplying the last value by 2.75.

Metabolic studies on man confirm the existence of a relationship between potassium and protein-enzyme. During the protein diet, the potassium intake must be high to equilibrate

Table 6. Urinary excretion of tetra-hydroaldosterone (mcg/24 h) in man during the protein diet (8 subjects)

											Mean ± SD
2nd day	72.0	74.5	78.0.	52.0	39.5	40.0	23.0	32.0	34.5	49.5	± 6.86
19th day	182.5	148.5	237.0	312.5	195.5	272.5	88.0	78.0	225.5	193	±26.4

the nitrogen balance. The mechanism seems to be following one: the sodium intake is lower than 1 meq/24 h and in these conditions an hypersecretion of aldosterone occurs (Table 4). Therefore, a high loss of potassium does take place. Il the potassium intake is not sufficient, the potassium pool decreases. Since potassium/protein-enzyme ratio must remain constant, such a condition leads to a negative nitrogen balance.

3. *Conclusions*

From metabolic experiments in rat and in human subjects submitted to different nutritional conditions, it can be concluded that:

- the exchangeable potassium is equal to the total potassium,
- the potassium/protein-enzyme ratio remains constant during aging and undernutrition,
- the negative potassium balance – caused by an endogenous hypersecretion of aldosterone following a low potassium intake – induces a negative nitrogen balance.

Bibliography

1. *Aikawa, J.K.:* Effect of starvation on exchangeable potassium and tissue ^{42}K content in rabbits. Proc. Soc. exp. Biol. N.Y., *78*, 524 (1951).
2. *Anderson, E.C.*, and *Langham, W.H.:* Average potassium concentration of the humany body as a function of age. Science *130*, 713 (1959).
3. *Apfelbaum, M., Baron, P., Bigot, R.*, and *Médici, A.:* Variation du potassium échangeable en fonction du poids chez le rat. C.R. Soc. Biol., Paris, *158*, 975–981 (1964).
4. *Apfelbaum, M.*, and *Brigant, L.:* Etude expérimentale de l'emploi de la mesure du potassium échangeable pour l'appréciation de la masse cellulaire chez le rat. J. Physiol. *62*, 1, 81–98 (1970) (a).
5. *Apfelbaum, M., Bostsarron, J.*, and *Delcroix, P.:* Le rôle du potassium dans le traitement de l'obésité par la diète protéique. Rev. franç. Diet. *47*, 44–48 (1970) (b).
6. *Apfelbaum, M., Boudon, P., Lacatis, D.*, and *Nillus, P.:* Effets métaboliques de la diète protidique chez 41 sujets obèses. Pres. med. *78*, 44, 1917–1920 (1970) (c).
7. *Apfelbaum, M.:* Traitement de l'obésité par la diète protéique. Ed. Exp. scienc. franç.: Entretiens de Bichat 62–64. Sem. thé. *44*, 3, 174–176 (1967). Influence du terrain et des thérapeutiques adjuvantes dans le traitement de l'obésité par la diète protéique. Ed. Exp. scien. franç.: Entretiens de Bichat 62–64. Sem. thé. *44*, 3, 174–176 (1968).
8. *Apfelbaum, M., Bostsarron, J., Brigant, L.*, and *Dupin, H.:* La composition du poids perdu au cours de la diète hydrique. Gastroenterologia *108*, 3, 121–134 (1967).
9. *Apfelbaum, M., Trémolières, J., Péquignot, E.*, and *Baron, P.:* Aspects physiologiques des dénutritions. VII Journées de Nutrition, Entretiens de Bichat. Les maigreurs, Expansion Scientifique, Paris, 17–48, 1965.
10. *Baldwin, D., Robinson, P.K., Zierler, K.L.*, and *Lilienthal, J.L.:* Interrelations of magnesium, potassium, phosphorus and creatine in skeletal muscle of man. J. clin. Invest. *31*, 850–858 (1952).
11. *Barnes, B.A., Gordon, E.B.*, and *Cope, O.:* Skeletal muscle analysis in health and in certain metabolic disorders. I. The method of analysis and the values in normal muscle. J. clin. Invest. *36*, 1239–1248 (1957).
12. *Boudon, P.:* Effets métaboliques et endocriniens de la diète protidique. Thèse Doct. Méd. Paris, juin, 168 pp., ronéotypée, 1969.
13. *Brigant, L.:* Technique d'appréciation nutritionnelle des matières grasses d'origine animale ou végétale. Thèse ing. Conserv. nat. Arts Métiers, Paris, 114 pp., ronéotypées, 1963.
14. *Cheek, D.B.*, and *West, C.D.:* Alterations in body composition with sodium loading and potassium restriction in the rat: the total body Na, N, Mg, Ca. J. clin. Invest. *35*, 763 (1956).

15. *Cier, J.F., Cier, A.*, and *Klepping, J.*: La répartition intra et extra-cellulaire des ions Na^+ et K^+ chez le rat. C.R. Soc. Biol., Paris, *152*, 1138–1140 (1958).
16. *Corsa, L., Olney, J.M., Steenburg, R.W., Boll, R.W.*, and *Moore, F.D.*: Exchangeable potassium measured by isotope dilution. J. clin. Invest. *29*, 1280 (1950).
17. *Davidson, D., McIntyre, I., Rapoport, A.*, and *Bradley, J.E.S.*: Determination of total fat *in vivo* with ^{85}Kr. Biochem. J. *62*, 34 (1956).
18. *Demanet, J.C.*: Mesure de l'échangeable, sodium et potassium, chez le rat. Rev. franç. Etud. clin. biol. *9*, 871 (1964).
19. *Edelman, I.S., Leibman, J., O'Meara, M.P.*, and *Birkenfeld, L.W.*: Interrelations between serum sodium concentration, serum osmolarity and total exchangeable sodium, total exchangeable potassium and total body water. J. clin. Invest. *37*, 1236–1256 (1958).
20. *Edwards, D.A.W.*: Observation on the distribution of subcutaneous fat. Clin. Sci. *9*, 259 (1950).
21. *Elkinton, J.R.*, and *Huth, E.J.*: Body fluid abnormalities in anorexia nervosa and undernutrition. Metabolism *8*, 376–403 (1959).
22. *Elkinton, J.R.*, and *Widdowson, E.M.*: Effect of chronic undernutrition on body composition in the rats. Metabolism *8*, 404–407 (1959).
23. *Fenn, W.O., Noonan, T.R., Mullins, L.J.*, and *Haege, L.*: Exchange of radioactive with body potassium. Amer. J. Physiol. *135*, 149 (1941).
24. *Ginsburg, J.M.*, and *Wilde, W.S.*: Distribution kinetics of intravenous radiopotassium. Amer. J. Physiol. *179*, 63 (1954).
25. *Green, R.M., McNeil, K.G.*, and *Robinson, G.A.*: The distribution of potassium and caesium 137 in the calf and the pig. Canad. J. Biochem. *39*, 1021 (1965).
26. *Greene, R.W.*, and *Sapirstein, L.A.*: Total body sodium, potassium and nitrogen in rats made hypertensive by subtotal nephrectomy. Amer. J. Physiol. *169*, 343 (1952).
27. *Harrison, H.E., Darrow, D.C.*, and *Yanet, H.*: The total electrolyte content of animals and its probable relation to the distribution of body water. J. biol. Chem. *113*, 515 (1936).
28. *Haxhe, J.J.*: La composition corporelle normale: ses variations au cours de la sous-alimentation et de l'hyperthyroïdie, Maloine, Paris, 345 pp., 1963.
29. *Huth, E.S.*, and *Elkinton, J.R.*: Effect of acute fasting in the rat on water and electrolyte content of serum and muscle and total body composition. Amer. J. Physiol. *196*, 299–302 (1959).
30. *Kirton, A.H.*: Relationship between potassium and body composition. Ann. N.Y. Acad. Sci. *110*, 221 (1963).
31. *Lemley-Stone, J.D., Darby, W.J.*, and *Meneely, G.R.*: Effect of dietary Na/K ratios in rats. Amer. J. Cardiol. *8*, 748 (1964).
32. Metropolitan Life Insurance Co: Ideal weights for men. Statist. Bull. *23*, 6–8 (1942); Ideal weights for women. Statist. Bull. *24*, 6–8 (1943).
33. *Moore, F.D., McMurrey, J.D., Parker, H.V.*, and *Magnus, I.C.*: Body composition; total body water and electrolytes: intravascular and extravascular phase volums. Metabolism *5*, 447–467 (1956).
34. *Noonan, T.R., Fenn, W.O.*, and *Haege, L.*: The distribution of injected radioactive potassium in rats. Amer. J. Physiol. *132*, 474 (1941).
35. *Norman, N.*: The participation of bone in Na + neous determination of the exchangeable body Na + K and the exchangeable fractions of these ione in bone in normal rats. Acta physiol. scand. *37*, 363 (1963).
36. *Smith, R.*, and *Waterlow, J.C.*: Total exchangeable potassium in infantile malnutrition. Lancet *1*, 147–149 (1960).
37. *Spray, C.M.*, and *Widdowson, E.M.*: The effect of growth and development on the composition of mamals. Brit. J. Nutr. *4*, 332 (1950).
38. *Talso, P.J., Miller, C.E., Carbella, A.J.*, and *Vasquez, I.*: Exchangeable potassium as a barometer of body composition. Metabolism *9*, 456 (1960).
39. *Waterlow, J.C., Craviota, J.*, and *Stephen, J.M.*: Protein malnutrition in man. Advanc. Protein Chem. *15*, 131–238 (1960).
40. *Waterlow, J.C.*, and *Mendes, C.B.*: Composition of muscle in malnourished human infants. Nature, Lond., *180*, 1361–1362 (1957).
41. *Waterlow, J.C.*, and *Weisz, T.*: The fat, protein and nucleic acid content of the liver in malnourished human infants. J. clin. Invest. *35*, 346–354 (1956).
42. *Widdowson, E.M.*, and *McCance, R.A.*: The effects of chronic undernutrition and of total starvation on growing and adult rats. Brit. J. Nutr. *10*, 363–373 (1956).
43. *Widdowson, E.M., McCance, R.A.*, and *Spray, c.M.*: The chemical composition of the human body. Clin. Sci. *10*, 113–125 (1951).

Interrelationships between Acid, Phosphorus and Potassium Excretion in the Urine of Sheep and Cattle

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Summary

Sheep and calves fed roughage diets excreted an alkaline urine poor in phosphorus. In the sheep infusion of acid into the rumen increased the excretion of acid in the urine and most of this was excreted as ammonium ions. Excretion of this acid did not reduce urinary excretion of potassium. In subsequent experiments intra-ruminal infusion of potassium chloride during acid infusion resulted in a marked rise in urinary potassium excretion but excretion of acid in the urine was not affected.

In contrast to animals fed roughage diets, sheep and calves fed a concentrate diet excreted an acid urine rich in ammonium ions and titratable acid phosphate. Intra-ruminal infusion of sodium bicarbonate although abolishing urinary acid excretion did not affect the excretion of potassium in the urine.

It is concluded that sheep and calves are able to excrete appreciable amounts of acid and potassium in the urine without evidence of competition.

1. *Introduction*

Acid is eliminated in the urine largely as a mixture of ammonium ions and titratable acid of which acid phosphate ($H_2PO_4^-$) is the major component. Excretion of acid has been studied in detail in man and dog and it has been shown that the rate of excretion of titratable acid is limited by the amount of available buffer excreted in the urine [*6, 8*]. It has also been suggested that, in dog, the rate of excretion of acid may be affected by the level of potassium in the diet. This is because it is believed that in the formation of an acid urine, hydrogen ions and potassium ions in the renal tubular cell may compete in an exchange process for sodium ions present in the tubular urine [*2, 3*].

Our interest in this system arises because sheep and cattle normally consume in their diets amounts of potassium which are considerably in excess of requirement and the surplus is excreted in the urine [*1, 4, 10*]. This may present little problem when roughage diets such as hay, straw or dried grass are fed for the excess potassium is excreted in an alkaline urine [*4, 9, 10, 11*].

A different situation may exist when concentrated foods are fed, for published reports indicate that cattle fed such diets excrete an acid urine rich in ammonium ions and titratable acid phosphate [*7, 11, 12*] and it is in these circumstances that competition might be expected to occur.

In view of this possible competition we decided to examine the relationship between acid and potassium excretion in sheep and calves fed either roughage or concentrate diets.

2. *Materials and Methods*

Four castrate Friesian calves, two Dorset-horned ewes and five Scottish-blackfaced ewes were used in these studies. At the beginning of the experiments the calves averaged 80 kg and the sheep 50 kg in weight. All of the sheep and two of the calves were fitted with a rumen cannula. During experiments the animals were kept in metabolism cages that allowed the separate collection of faeces and urine.

In some experiments increased excretion of acid or potassium in the urine was achieved by infusing differing amounts of hydrochloric acid or potassium chloride into the rumen while in other experiments reduced excretion of acid in urine was achieved by infusing sodium bicarbonate into the rumen. The amounts of these differing substances infused are given in the text. The procedures for collecting and storage of samples and the chemical methods of analysis have been previously described [*10, 11*]. Throughout these experiments the method of presenting net acid excretion in urine is that described by *Jørgensen* [*5*] in which net acid excretion

$$= H^+_{NH_4^+} + H^+_{\text{titr. acid}} - HCO_3^- - OH^-_{\text{titr. base}}.$$

3. *Results*

3.1 *Roughage diets*

Figure 1 shows the effects of infusing 200 mmole/day of hydrochloric acid into the rumen of two sheep fed 800 g/day of a pelleted grass diet supplying 360 mmole of potassium. In the control period the urine was alkaline (pH 8.1—8.7) and in response to acid infusion urine pH declined and excretion of acid in the urine rose. Excretion of potassium in the urine was unaffected by acid infusion and it is clear that sheep can excrete appreciable amounts of acid and potassium in the urine at the same time. The results shown in Figure 2 are from another experiment of this type in which a detailed analysis was made of the chemical forms in which the acid was excreted. In this experiment 200 mmole/day of hydrochloric acid was infused into the rumen of a sheep fed the pelleted grass diet. Before infusion of acid the urine was again alkaline (pH 8.1—8.6) and between −73 and −45 mmole of net acid were excreted in the urine each day. In response to the infusion of acid the pH of the urine declined and excretion of acid rose. Analysis of this acid revealed that most of it was present as ammonium ions in the urine. Excretion of phosphorus in the urine during the experiment was low, averaging 0.4 g/day, and because of this there were only small amounts of titratable acid phosphate present in the urine during acid infusion. As in the previous experiment excretion of potassium in the urine was unaffected by acid infusion.

The results shown in Figure 3 illustrate the effects on acid and potassium excretion of varying the level of potassium in the diet during acid infusion. In this experiment the two sheep were fed a diet which contained 53.2% barley straw, 10.6% maize starch, 32.0% groundnut meal, 2.0% molasses and 2.2% of a vitamin-mineral mixture. This diet, fed at a rate of 800 g/day provided 110 mmole of potassium.

Infusion of 150 mmole/day of hydrochloric acid into the rumen led to a rise in the rate of excretion of acid in the urine. Excretion of potassium in the urine was low in response to

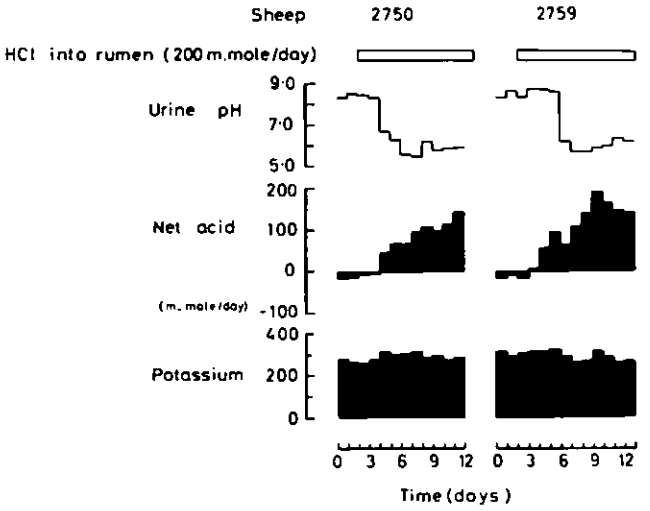

Figure 1. The effects of intra-ruminal infusion of HCl upon the urinary excretion of net acid and potassium in sheep. Dietary potassium intake was 360 mmole/day.

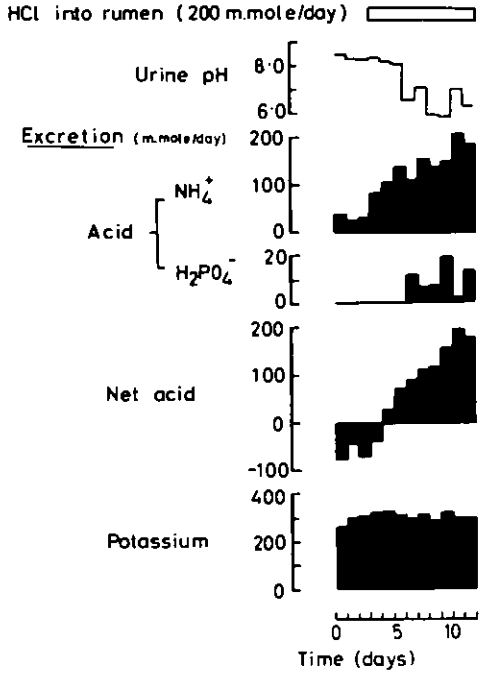

Figure 2. The effects of intra-ruminal infusion of HCl upon some components of urinary acid excretion. Dietary potassium intake 360 mmole/day.

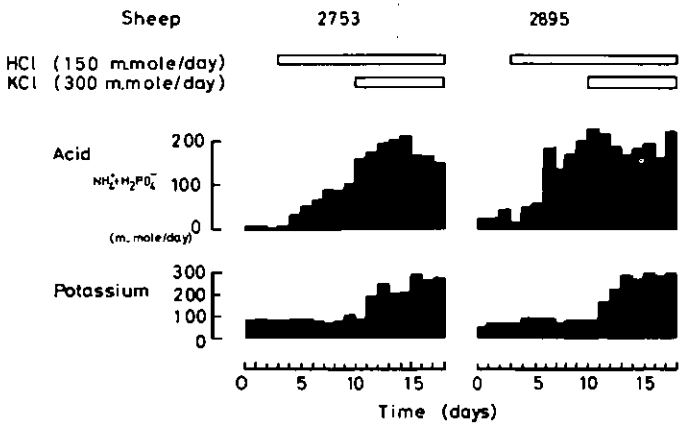

Figure 3. The effects of varying potassium intake during acid infusion in sheep. Dietary potassium intake was 110 mmole/day.

the low potassium intake and was not affected by acid infusion. A supplement of 300 mmole/day of potassium chloride which increased the potassium intake up to that seen in sheep fed the pelleted grass led to a rise in the rate of excretion of potassium up to that seen in sheep fed the pelleted grass diet but the supplement did not affect the excretion of acid in the urine. As before most of the acid excreted was in the form of ammonium ions with only negligible amounts of titratable acid phosphate being excreted.

3.2 *Concentrate diets*

The results shown in Table 1 illustrate the differences in urine pH and acid excretion in sheep and calves fed a roughage as opposed to a concentrate diet. The roughage diet used in these experiments was the same as used in the experiments shown in Figure 3, while the concentrate diet contained 86.0% bruised barley, 12.0% fish meal and 2.0% of a vitamin-mineral supplement. These two diets were about equal in potassium content.

The results of these experiments show that when both sheep and calves were fed a roughage diet they excreted an alkaline urine poor in phosphorus whereas when they were fed the concentrate diet the urine was acid and contained large amounts of phosphorus.

Table 1. Renal excretion of acid and potassium in two sheep and four calves fed either a roughage or a concentrate diet. Mean values for a 6-day collection period ± S.D.

	Diet	Food intake	Urine pH	Phosphorus	Acid		Potassium
					Titratable $H_2PO_4^-$	NH_4^+	
		kg		g	mmole	mmole	
Sheep	Roughage	1.4	8.2 ± 0.7	0.3 ± 0.2	0.3 ± 0.2	36 ± 13	163 ± 38
	Concentrate	1.4	5.6 ± 0.2	5.5 ± 1.1	164.1 ± 37.0	167 ± 20	164 ± 26
Calves	Roughage	2.0	7.7 ± 0.2	0.6 ± 0.2	3.0 ± 2.1	36 ± 16	217 ± 48
	Concentrate	2.0	6.0 ± 0.2	5.7 ± 1.4	159.5 ± 37.4	163 ± 52	198 ± 42

There were also marked differences in urinary acid excretion when the different diets were fed. Thus appreciable amounts of both titratable acid phosphate and ammonium ions were excreted in the urine when the concentrate diet was fed but only small amounts of titratable acid phosphate and ammonium ions were excreted when the roughage diet was fed. There was no evidence that the amount of potassium excreted in the urine was in any way reduced in the presence of the large amounts of acid excreted when the concentrate diet was fed compared to when the roughage diet was fed. This relationship between urinary excretion of acid and potassium was examined further in a sheep and a calf fed 1.4 and 2.0 kg/day of the concentrate diet respectively (Figure 4). In a 6-day control period excretion of acid in the urine of the sheep averaged 295 mmole/day and excretion of phosphorus averaged 6.0 g/day of which 92% (183 mmole) was excreted as titratable acid phosphate. In the calf excretion of acid in the control period averaged 403 mmole/-day and excretion of phosphorus averaged 6.7 g/day of which 85% (195 mmole were excreted as titratable acid phosphate. At the end of the control period 300 and 450 mmole/day of sodium bicarbonate were given as a continuous infusion into the rumen of the sheep and the calf respectively. This was continued for nine days and led to a rise in urine pH and to a fall in urinary acid excretion. Excretion of phosphorus in the urine continued at the same rate as in the control period but only a small proportion was excreted as titratable acid. Excretion of potassium during the experiment averaged 174 and 208 mmole/day in the sheep and the calf respectively and there was no change in excretion rate in response to the change in net acid or titratable acid excretion during bicarbonate infusion.

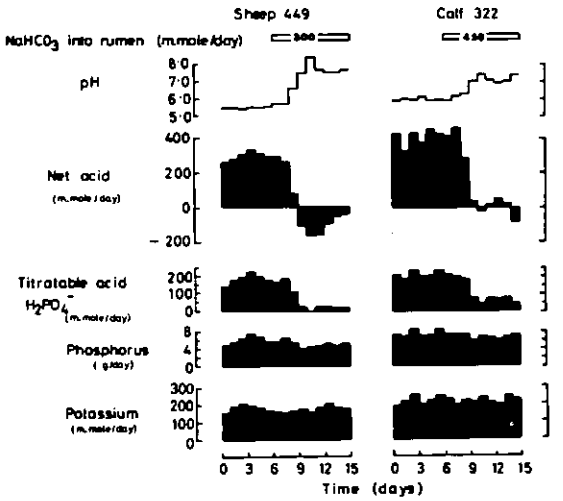

Figure 4. The effects of intra-ruminal infusion of sodium bicarbonate on the urinary excretion of acid and potassium in a sheep and a calf fed a concentrate diet.

4. *Conclusions*

The results of these experiments show that infusion of acid into the rumen of sheep results in the excretion of large amounts of acid in the urine but this had no effect on the amount of potassium excreted in the urine. Conversely the infusion of large amounts of potassium into the rumen during the infusion of acid did not limit the capacity of the kidney for acid excretion.

In control periods both sheep and calves fed roughage diets excreted an alkaline urine which contained only small amounts of phosphate. In consequence, during acid infusion, acid excreted in the urine appeared mostly as ammonium ions rather than as titratable acid phosphate.

In contrast, sheep and calves fed a concentrate diet excreted an acid urine and this contained both large amounts of ammonium ions and titratable acid phosphate. There was, however, no evidence that the elimination of these large amounts of titratable acid in any way reduced the proportion of dietary potassium in the urine, for about equal amounts of potassium were excreted in the urine when the roughage or the concentrate diets were fed, and the diets themselves were about equal in potassium content. In addition, the infusion of bicarbonate into the rumen of a sheep and a calf fed the concentrate diet, although largely abolishing the excretion of acid in the urine, produced no change in the rate of excretion of potassium.

In conclusion, sheep and calves appear to be able to excrete large amounts of potassium and acid in the urine without evidence of competition.

Bibliography

1. *Anderson, R.S.*, and *Pickering, E.C.*: Effects of intravenous infusion of potassium chloride on potassium and sodium excretion and on the rate of urine formation in the cow. J. Physiol. *164*, 180 (1962).
2. *Berliner, R.W., Kennedy, T.J.*, and *Hilton, J.G.*: Renal mechanisms for the excretion of potassium. Amer. J. Physiol. *162*, 348 (1950).
3. *Berliner, R.W.*: Renal secretion of potassium and hydrogen ion. Fed. proc. *11*, 695 (1952).
4. *Dewhurst, J.K., Harrison, F.A.*, and *Keynes, R.D.*: Renal excretion of potassium in the sheep. J. Physiol. *195*, 609 (1968).
5. *Jørgensen, K.*: Titrimetric determination of the net excretion of acid-base in urine. Scand. J. clin. lab. Invest. *9*, 287 (1957).
6. *Pitts, R.F., Lotspeich, W.D., Schiess, W.A.*, and *Ayer, J.L.*: The renal regulation of acid-base balance in man. I. The nature of the mechanism acidifying the urine. J. clin. Invest. *27*, 48 (1948).
7. *Reed, W.D.C., Elliot, R.C.*, and *Topps, J.H.*: Phosphorus excretion of cattle fed on high energy diets. Nature (Lond.) *208*, 953 (1965).
8. *Schiess, W.A., Ayer, J.L., Lotspeich, W.D.*, and *Pitts, R.F.*: The renal regulation of acid-base balance in man. II. Factors affecting the excretion of titratable acid by the normal human subject. J. clin. Invest. *27*, 57 (1948).
9. *Scott, D.*: The effects of variations in water or potassium intake on the renal excretion of potassium in sheep. Q. Jl. exp. Physiol. *54*, 16 (1969).
10. *Scott, D.*: Renal excretion of potassium and acid by sheep. Q. Jl. exp. Physiol. *54*, 412 (1969).
11. *Scott, D., Whitelaw, F.G.*, and *Kay, M.*: Renal excretion of acid in calves fed either roughage or concentrate diets. Q. Jl. exp. Physiol. *56*, 18 (1971).
12. *Topps, J.H., Reed, W.D.C.*, and *Elliot, R.C.*: Studies of the metabolism of cattle given high concentrate diets. J. agric. Sci. *66*, 233 (1966).

Effects of Calcium on Resorption and Excretion of Major and Some Minor Elements in Cattle

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Summary

The effects of calcium supplementation on availability and excretion pattern of major elements and some minor elements was studied in balance trials with lactating identical twins. A daily dose of 160 g $CaCO_3$ did not affect digestion of dry matter, organic matter and nitrogen; digestion of higher fatty acids was insignificantly decreased. Availability of K, Na, Mn and P was significantly increased by calcium supplementation, whereas availability of Cl and Ca was decreased. The balance of K, Na and Cl, however, was not changed because of a compensatory effect on the urinary excretion.
Retention of Ca, Mn and P was increased by the treatment; secretion of I is in the milk decreased. There was no treatment effect on the excretion patterns of S, Zn and Cu.

1. *Introduction*

Potassium fertilizing and the potassium status of the soil affect the mineral composition of the grass to a great extent *(Dijkshoorn [6, 7]; Hemingway [11]* and *Kemp [13]).* Under field conditions in the Netherlands a potassium application will hardly affect the botanical composition of the sward. The effect on the cation composition of the harvested crop, however, may be considerable.
From the fertilizer experiments over many years of *Kemp* and *Geurink [15]* it can be calculated that by increasing the fertilizer application from 122 kg K_2O to 319 kg K_2O per ha the Ca content in the grass shows an average decrease from 0.66% to 0.51%. In intensive cattle farming there is a risk of the calcium supply to the cattle growing marginal in this way.
The effect of potassium supplementation of the rations on the retention and excretion pattern of major elements in cattle and sheep has been treated in a number of studies *(Kemp et al. [14]* and *Van Koetsveld [16]).* In the field, however, we also have to deal with the effect on the animal of the changed chemical composition of the plant induced by potassium application.
In the present experiments the effect was studied of the calcium content in the rations – calcium being a cation much affected by potassium application – on the resorption and excretion by the cow of major and some minor elements.

2. *Material and Methods*

Three sets of Friesian identical twins were divided into two groups, in such a way that one of the twins was in the first and the other in the second group. These animals were used in a reversal balance trial.

The cows were in the second part of their first or second lactation period and their average weight was 503 kg. The basal ration consisted of 9.33 kg pasture hay, 2.67 kg ground maize and 1.08 kg cotton seed meal and was supplied twice daily. There were small refusals and only from the hay; during collection periods they averaged 0.12 kg per animal per day. One group received the basal ration, for the other group 160 g additional calcium carbonate was mixed in the concentrates. The animals were equipped with harnasses for separate collection of faeces and urine after *van Es* and *Vogt* [9]. The faeces dropped on stainless steel trays and the urine was collected in large polyvinylchloride (PVC) flasks.

After a 14-day preliminary period to get the animals adjusted to the rations, a collection period of 8 days followed. During this period the feed consumed, feed refusals, collected faeces, urine and milk of each cow were carefully weighed and duplicate or triplicate proportional samples were stored in PVC pots. Immediately after the first collection period the treatments were changed. During an 8 day preliminary period samples were collected on the second and fifth day, after which a second collection period of 8 days followed.

The samples from the collection periods were analysed for contents of dry matter (DM), organic matter (OM), higher fatty acids (HFA) *(Immink et al. [12])*, N, K, Na, Mg, Ca, P, Cl, S, Zn, Mn, and Cu. Milk samples from both collection periods and from the second and fifth day of the second preliminary period were analysed for I. Statistical analyses were made after the methods of *Snedecor* [18] for factorial design.

3. *Results and Discussion*

The $CaCO_3$ supplementation increased the Ca content in the dry matter of the rations from 0.49% to 1.09% and the Ca:P ratio from 1.2 to 2.7. The calculated intake and excretion as well as the mineral balance and the availability or digestibility of the different constituents in both treatment groups are presented in table 1.

As a result of the calcium carbonate supplementation the dry matter content of the faeces is significantly decreased; by increased drinking the cows compensate the greater water excretion induced in this way.

The digestion of dry matter, organic matter and crude protein is not affected by the $CaCO_3$ supplementation. In the literature some cases have been described of an adverse effect of $CaCO_3$ supplementation on the digestibility of dry matter, crude protein, energy or energy value determinants in cattle *(Ammerman et al. [1]; Colovos et al [5]; Dowe et al. [8])*. Except for the experiments of *Colovos et al.* [5], in all the experiments in which a $CaCO_3$ dose, relative to the dry matter intake, was used comparable to ours, no clear effects were achieved. The effects described by *Colovos et al.* may also have been induced by a possibly insufficient P supply.

The digestion of higher fatty acids shows an insignificant decrease in the high calcium animals. This may have been the result of the formation of more insoluble Ca soaps in the digestive tract *(Cheng et al. [4])*.

Table 1. Intake, excretion, balance and availability (digestibility) of dry matter, organic matter, higher fatty acids, nitrogen and minerals in cows on calcium carbonate supplemented or unsupplemented rations.

Per cow per day	Intake		Excretion in: faeces		urine		milk		Balance		Availability (digestibility) %	
	Ca	B	Ca	B	Ca	B	Ca	B	Ca	B	Ca	B
DM, kg	11.10a	10.94b	3.33	3.26	—	—	—	—	—	—	70	70
OM, kg	10.00	10.00	2.70	2.70	—	—	—	—	—	—	73	73
HFA, me	920	920	266	242	—	—	—	—	—	—	71	74
N, g	250	250	89	90	—	—	—	—	—	—	64	64
K, g	263	263	20e	46f	228c	200d	17	17	− 2	+ 0	92c	83d
Na, g	9.3	9.3	1.4c	2.8d	2.4c	0.7d	3.9	3.8	+ 1.6	+ 1.9	85c	69d
Cl, g	71.2	71.2	11.2c	6.6d	47.3c	51.3d	10.4	10.2	+ 2.4	+ 3.0	84e	91f
Mg, g	21.4	21.2	16.3	15.8	2.4a	2.6b	1.1	1.1	+ 1.6	+ 1.7	25	26
Ca, g	120.9	53.4	100.9e	42.3f	0.7a	0.5b	12.8	12.9	+ 6.4c	− 2.2d	16a	21b
P, g	45.1	45.1	32.8	33.7	0.2	0.3	9.9	9.9	+ 2.2a	+ 1.3b	27c	25d
S, g	29.2	29.0	12.4	11.9	10.0	9.8	3.3	3.4	+ 3.5	+ 3.9	58	59
Zn, mg	330	329	323	316	1.4	0.9	34	37	−28	−24	2	4
Mn, mg	251	247	227c	244d	0.0	0.0	0.2	0.2	+24c	+ 3d	10c	1d
Cu, mg	74.6	74.1	75.1	74.7	0.2	0.2	0.2	0.2	− 1.0	− 1.0	− 0.7	− 0.7
I, mg	—	—	—	—	—	—	0.42a	0.48b	—	—	—	—
DM %			14.7a	15.7b								
kg produced (fresh)			22.66a	20.96f	15.22	15.26	9.97	9.88				
water intake, kg	47.6	45.9										

B = basal ration; Ca = $CaCO_3$ supplemented ration; — = not determined
Values without superscript are not significantly different; superscripts a and b indicate: $P < 0.05$ c and d indicate: $P < 0.01$ e and f indicate: $P < 0.001$

On the high calcium ration the availability of K and Na is highly increased, whereas that of Cl is decreased. The higher resorption of the cations is associated with an increased excretion in the urine, thus leaving the balance unaffected. The Cl balance is also unaffected, but by a lower urinary excretion. These findings are in accordance with the generally accepted view that excess of these minerals is excreted in the urine, and that in case of shortage urinary excretion is decreased.
As to Mg, a similar tendency as in K and Na is apparent; the difference between treatments, however, is very small and does not seem to be of practical significance.
The increased Ca intake results in a decreased Ca availability. Nevertheless, the negative Ca balance on the basal ration turns into a definitely positive one in the supplemented group. At the same time the low urinary excretion increases slightly. In our experiments, in which Ca supply will have been near marginal, an availability of 13% can be calculated for the supplemental Ca. On the contrary, *Haag et al.* [*10*] with cows on lucerne rations with a more liberal Ca supply (1.16% Ca and 0.153% P) found no demonstrable utilization of supplemental $CaCO_3$.
The availability and the balance of P are slightly, but significantly increased by Ca supplementation. Presumably, the increased Ca retention stimulates P retention, as in general both elements are simultaneously required by the animal, e.g. for bone formation or for lactation.
The availability of Mn is highly increased by the Ca supplementation, resulting in a significantly higher retention in the animal. Though Ca long has been considered as a factor depressing Mn adsorption, our results are in line with recent experiments by *Lassiter, Morton* and *Miller* [*17*] with radio-active and stable Mn in rats. As the animals on the basal ration already were in a slightly positive Mn balance, these findings also lead to the question to what extent these and other supplementations may affect tissue Mn concentrations.
There is no treatment effect on distribution of excretions of S, Zn, and Cu. The marked negative Zn balance in both treatments is somewhat embarrassing. It is hardly possible to suspect Zn contamination in faeces as, e.g. the urine analyses did not show substantial quantities of Zn. Since all the samples were stored in the same type of pots obtained from the same supplier contamination seems unlikely.
The I secretion in the milk is distinctly decreased by $CaCO_3$ supplementation. Figure 1 shows the I concentration in the milk during the experiments. It can be seen that 2 days after change in treatments the effect on the I content in the milk is apparent. After that there is a rise in both groups, presumably because milk production showed a tendency to decrease. Since the I secretion in the milk is considered as an indication of the I supply to the animal in a short preceding period *(Binnerts* [*2*]*)*, this result may be regarded as a distinct indication that $CaCO_3$ decreases the I retention. According to *Boyle et al.* [*3*] this effect of Ca may be caused in man by interference in the absorption of I from the gut.

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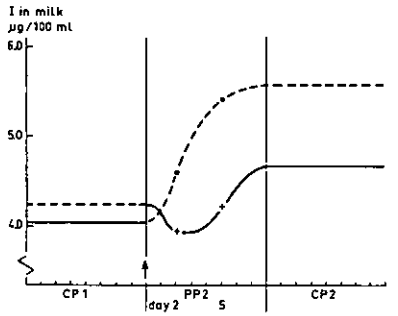

----- basal ration
_____ with $CaCO_3$ supplementation
PP = preliminary period CP = collection period
↑ indicates change of treatments

Figure 1. Iodine concentration in the milk as influenced by change of treatments.

Bibliography

1. *Ammerman, C.B., Arrington, R.L., Jayswal, M.C., Shirley, R.L.,* and *Davis, G.K.:* Effect of dietary calcium and phosphorus levels on nutrient digestibility by steers. J. Anim. Sci. *22*, 248 (1963).
2. *Binnerts, W.T.:* Het jodiumgehalte van melk. Thesis Univ. of Amsterdam, 1956.
3. *Boyle, J.A., Greig, W.R., Fulton, S.,* and *Dalakos, T.G.:* Excess dietary calcium and human thyroid function. J. Endocrinol. *34*, 531–532 (1966).
4. *Cheng, A.L.S., Morehouse, M.G.,* and *Deuel, Jr., H.J.:* The effect of the level of the dietary calcium and magnesium on the digestibility of fatty acids, simple triglycerides and some natural and hydrogenated fats. J. Nutr. *37*, 237–250 (1949).
5. *Colovos, N.F., Keener, H.A.,* and *Davis, H.A.:* Effect of pulverized limestone and dicalciumphosphate on the nutritive value of dairy cattle feed. J. Dairy Sci. *41*, 676–682 (1958).
6. *Dijkshoorn, W.:* Nitrogen, chlorine and potassium in perennial ryegrass and their relation to the mineral balance. Neth. J. agric. Sci. *6*, 131–138 (1958).
7. *Dijkshoorn, W.:* The balance of uptake, utilization and accumulation of the major elements in grass. Potassium in relation to grassland production. Proc. First Reg. Conf. Intern. Potash Inst., Wexford (Ireland) 43–62, 1963.
8. *Dowe, T.W., Matsushima, J.,* and *Arthaud, V.H.:* The effects of adequate and excessive calcium when fed with adequate phosphorus in growing rations for beef calves. J. Anim. Sci. *16*, 811–820 (1957).
9. *Es, H.J.A. van,* and *Vogt, J.E.:* Separate collection of feces and urine of cows. J. Anim. Sci. *18*, 1220–1223 (1959).
10. *Haag, J.R., Jones, I.R.,* and *Brandt, P.M.:* The influence of various mineral supplements on the calcium, phosphorus and nitrogen metabolism of dairy cattle. J. Dairy Sci. *15*, 23–28 (1932).
11. *Hemingway, R.G.:* Magnesium, potassium, sodium and calcium contents of herbage as influenced by fertilizer treatment over a three-year period. J. Brit. Grassl. Soc. *16*, 106–116 (1961).
12. *Immink, H.J., Geurink, J.H.,* and *Deijs, W.B.:* The determination of the higher fatty acids in grass and cow-faeces. Jaarb. Inst. Biol. Scheik. Onderz. LandbGew. 103–107, 1965.
13. *Kemp, A.:* Hypomagnesaemia in milking cows: The response of serum magnesium to alterations in herbage composition resulting from potash and nitrogen dressings on pasture. Neth. J. agric. Sci. *8*, 281–304 (1960).
14. *Kemp, A., Deijs, W.B., Hemkes, O.J.,* and *Es, A.J.H. van:* Hypomagnesaemia in milking cows: intake and utilization of magnesium from herbage by lactating cows. Neth. J. agric. Sci. *9*, 134–149 (1961).
15. *Kemp, A.,* and *Geurink, J.H.:* Invloed van magnesiumbemesting op het magnesiumgehalte van de grond, het gras en het bloedserum bij melkkoeien. Versl. landbouwk. Onderz. 747 (1970).
16. *Koetsveld, E.E. van:* De invloed van verhoogde kaliumopname op de electrolyten-huishouding van verse melkkoeien. Tijdschr. Diergeneesk. *89*, 590–604 (1964).
17. *Lassiter, J.W., Morton, J.D.,* and *Miller, W.J.:* Influence of manganese on skeletal development in the sheep and rat. In: Mills C.F. (ed.): Trace element metabolism in animals, pp 130–132, Livingstone Edinburg-London, 1970.
18. *Snedecor, G.W.:* Statistical methods (5th ed.). Iowa State Univ. Press, Ames 1956.

Discussion, Session No. 3

Prof. Dr. *M. Kirchgessner* (Munich/Federal Republic of Germany):

Can you compare the frog skin with the intestine? If you stop the shunting effect of the anions with copper, have you
a) differences with different Cu-concentration?
b) Can you stop this effect also with other cations?

Dr. *V. Koefoed-Johnsen* (Copenhagen/Denmark):

I do think that frog skin and intestine are comparable with regard to salt transporting properties. Both tissues perform transepithelial active transport of sodium, and the underlying principles discussed in relation to the frog skin are also valid for the gut.
With respect to a) and b): These problems have not been studied in details, but are presently under investigation in our laboratory.

Dr. *V. Koefoed-Johnsen* (Copenhagen/Denmark):

Aldosterone is known to have a stimulating effect on Na-transport in toad bladder, toad- and frog-skin. A morphological study made by *Voûte, Dirix, Nielsen* and *Ussing* has shown that after addition of aldosterone the shape of the mitochondria-rich cells in *str. granulosum* becomes pear like with the apex reaching the subcorneal space which is being filled with some amorphous material. After 2–4 hours the cornified layer is completely detached from the underlying cell layer. When the cornified layer is ruptured or mechanically removed the short circuit current raises to values above the control levels. *Nielsen* has furthermore shown that both the moult and the activation of the short circuit current are abolished by actinomycin D. [ref.: Expl. Cell Res. *57*, 448 (1970), and Ac. Ph. Soc. *77*, 85 (1969)].

Dr. *L. Perlemuter* (Créteil/France):

To the remark of Mrs. *Koefoed* I can say of course that the only prove we have is that aldosterone acts directly on the sodium efflux-influx mechanism and that the K leak is a consequence of it. We have no prove of aldosterone action on K^+ transport mechanism.

Prof. Dr. *M. Kirchgessner* (Munich/Federal Republic of Germany):

In experiment with cattle we found an increased excretion of Fe, Zn and Mn at 10° and 30 °C environmental temperature in comparing with 20 °C. Can you discuss these results with biological rhythms?

Dr. *A. Reinberg* (Paris/France):

I have no reference concerning the effects of changes in environmental temperature on circadian rhythms in cattle. Several circadian rhythms were reported to persist during hibernation in some hinbernating mammals *(Ch. Kayser, H. & M.C. Saint-Girons, M.G. Kolpakov).* Circadian rhythms in a set of physiologic functions also persist in man during prolonged isolation in cave with low and constant environmental temperature (6° C $\pm$ 0.5 in our experiments with *F. Halberg, J. Ghata et al.*).
We have also to consider cyclical changes in envrionmental temperature with a period about 24 h, about a year, etc.) which, in some circumstances and/or for some animal species can play the role of a synchronizer.

Dr. *L.K. Wiersum* (Haren-Groningen/Netherlands):

Although plant has an endogenic rhythm it does not seem important in K-metabolism.
K-metabolism and movement seem more strictly related to the type of energy available at a certain time.
It is clear that the effect and type of energy available differs greatly between day and night (photoenergy and respiration energy mixed or respiration only), but these might not follow a special rhythm in lasting light or darkness. Intake by the root is very sensitive to a continuous supply of carbohydrate.

Prof. Dr. *I. Arnon* (Rehovot/Israel):

to *Dr. Reinberg:* a) Does the disruption in rhythm due to transatlantic flight have deleterious effects, and if yes – is there an effective therapy.
b) What is the situation regarding subjects, such as pilots, who fly back and forth over the Atlantic?

Dr. *A. Reinberg* Paris/France):

Changes in parameters characterizing circadian rhythms of human biological and physiological functions can be detected objectively after what we use to call an intercontinental flight. Under these conditions the organisms can be submitted to the influence of a shift in environmental factors after crossing several time zones. The expression transmeridian flight is more specific than intercontinental or long distance flight if this type of shift is being considered. A number of environmental factors with cyclical variations of about 24 h are effective in the synchronization of circadian rhythms; in the human subject socio-ecological factors related to societal life (timing of the alternation of diurnal activity and night rest) can be considered as a very powerful synchronizer.
After a transmeridian flight, when staying in a 'new' geographic location – associated with societal changes – for a certain timespan (several days, weeks, months, etc.), the subject is exposed to a phaseshift influence of the synchronizer; in these circumstances both the period of the synchronizer and the period of the biorhythms remain unchanged (24 h as mean).
From objective analyses of temporal series *(F. Halberg, A. Reinberg et al.)* one can conclude that the duration of resynchronization after a transmeridian flight:
1) varies from subject to subject for a given function;

2) varies, for a given subject, from function to function (i.e. sleep/wakefulness rhythm could be resynchronized within a few days, while adrenal activity rhythm – as reflected by urinary excretion of 17-hydroxycorticosteroids and potassium – will take several weeks to reset);
3) duration of resynchronization for a physiological function in an individual varies with direction of flight: long adaption usually corresponds to West to East flight, and fast adaption usually corresponds to East to West flight. A transmeridian flight could be followed by an alteration of phase-relationships of circadian rhythms; these phase-relationships characterize, at least, in part, the circadian temporal structure of each organism and species. These results must not be generalized from one subject to another, different physiological functions or other transmeridian flights. Therefore, it is obvious that new experiments, with standardized conditions of study and analysis, have to be done.

Prof. Dr. *J. Arnon* (Rehovot/Israel):

to *Dr. Perlemuter:* Do physicians who prescribe drugs that increase K^+ losses take this into account by presenting corrective measures?

Dr. *L. Perlemuter* (Créteil/France):

Yes, doctors know very well the effects of drugs on K^+ losses and of course the side-effects of drugs must be taken in account when treatment is given (especially long term treatment).
It is practically sure that commonly even when K is given to overcome the losses, it is not always given in sufficient quantities, and we do not know precisely the long term effects of K^+ deficiency.

Dr. *L. Perlemuter* (Créteil/France):

Are there some substances, produced by plants, similar to hormones, that can enhance potassium transfer?

Prof. Dr. *K. Mengel* (Hannover/Federal Republic of Germany):

Until now we do not know such substances, but we have to assume that in plants too, K specific organic substances exist.

Dr. *T. Walsh* (Dublin/Ireland):

1) The differences shown between roughages and concentrates in relation to urine pH raise the question of relationships to the excretion of Mg and Ca. Has Dr. *Scott* any information on this matter?
2) Has Dr. *Perlemuter* found any relationship between K and diabetic effects? What is the status of chemism in this connection. What is the status of blood K in relation to transfusion especially in the case of infants?

Dr. *D. Scott* (Aberdeen/Scotland):

Diurnal changes in urine pH do influence the rates of excretion of some minerals in urine. Australian workers have reported that soon after feeding in sheep fed once per day there is a transitory fall in urine pH and this is associated with increases in the rate of excretion of Ca^{++} and Mg^{++}. We also have found in calves and in deer that during infusion of HCl into rumen increased excretion of calcium in urine was closely correlated with a fall in urine pH.

Dr. *L. Perlemuter* (Créteil/France):

1) In diabetic condition K^+ has absolutely no biological influence. But in diabetic coma where there is an osmotic diuresis and acidosis both of them cause a renal loss of K^+. On the other hand where one gives insulin the glucose penetrates in the cells accompanied with great quantities of K^+. The consequence is a dramatic deficiency in circulating K^+ which must be prevented by administration of potassium salts in the preceeding period of treatment.
2) In blood transfusion there is no possibility of overaccumulation of K^+, unless if the patient has a disease involving the kidney and receives very high quantities of blood (blood has only 5 meq of K^+ per liter, which is a physiologic quantity).
3) Potassium to my knowledge has been poorly studied in this field. Anyway, the main problem is genetic, concerning the synthesis and release of insulin. Whether potassium has an influence on those stages has not been determined very precisely.

Prof. Dr. *G. Pulss* (Kiel/Federal Republic of Germany):

In our experiments on the metabolism of the dairy cows we have not observed the same results as did Mr. *Scott*. During the feeding of roughages we found a high pH-value in the urine, but a low level of NH_4 and of titratable P_2O_5. After raising the concentrates (barley) – and that is the difference to the findings of Mr. *Scott* – the pH-value remained also above 5.0 whereas – similarly as in the experiments of Mr. *Scott* – the levels of NH_4 and titratable P_2O_5 increased.
A question: Have you determined the sodium in the urine? Other experiments have shown, that the levels of Na^+ and NH_4^+ was lifted, when the pH-value falls, without any change in the content of titratable P_2O_5. This behaviour varied for the different cows receiving the same diet.

Dr. *D. Scott* (Aberdeen/Scotland):

We also have been interested in the relationship between urinary phosphorus excretion and pH in sheep and calves fed concentrate diets and observed that, when a diet containing 86% barley and 12% fish meal was fed, the urine pH was acid and contained large amounts of phosphate. In contrast, if we substituted soya bean meal for fish meal as the protein source we, like Prof. *Pulss*, found that the animals excreted an alkaline urine rich in phosphate. The reason for this difference in urine pH following this substitution of protein source is not yet clear.

Prof. Dr. *M. Kirchgessner* (Munich/Federal Republic of Germany):

Your results for the interaction Ca:Cu are in contrary to the 3 experiments of *Dick* with sheep and to our experiments with cattle. Increasing the Ca content of the food considerably diminished Cu retention. The reasons we derived from *in vitro* experiments is that the speed of dialysis of Cu is constant in acid ranges up to pH 5.5. With decreasing concentration of hydrogen ions the dialysis rate decreases very much. Have you an explanation why in your experiment there is no such an influence?

Mr. *J. Hartmans* (Wageningen/The Netherlands):

In the literature there is a discrepancy with respect to the effect of Ca on Cu metabolism in ruminants. I mention work of *Deys et al.* (1956), showing a positive correlation between Ca in the ration and Cu status of cattle. In own experiments (*Hartmans* and *van der Grift*, 1964) there was no difference in Cu content in the liver between groups of heifers on rations supplemented with equimolar quantities of $CaSO_4$ or Na_2SO_4. *Huber* neither found an influence of $Ca_3(PO_4)_2$ on liver Cu content in cattle. May be the different results will be due to the different Ca levels in the basal rations – which in the Dutch experiment were low – having different effects on ruminal pH or on other factors involved in Cu absorption (compare: *Suttle* and *Field:* Proc. Nutrit. Soc. *29*, 33A (1970).

Prof. Dr. *M. Kirchgessner* (Munich/Federal Republic of Germany):

I believe the difference is due to a Cu-deficiency in your ratio and a high protein level. Therefore you have a good absorption of Cu and no chemical reaction in the gut you will have between Ca and Cu.
Another question: The plant nutritionists use the expression availability. You take a synonyme to digestibility. We would like to reserve available to the intermediary metabolism after the absorption. We found in the last time big differences in the availability of trace elements in the metabolism after the absorption, differences caused by the bindings to different ligands.

Co-ordination Lecture for Session No. 3

Prof. Dr. R. BACH, Institute of Agricultural Chemistry, Federal Institute of Technology (ETH), Zürich, Switzerland; Member of the Scientific Board of International Potash Institute

Physiological research starts from observations, from facts measured or proved when possible with objective methods. Many observations or facts are apparently related; statistics allow to prove correlations.
Mr. *Reinberg* has shown with statistical methods, that potassium metabolism of man – as many other physiological processes – follows definite biological rhythms, i.e. it shows regular periodic changes as a function of time. Such rhythms may be considered as

genetically fixed; they may be influenced but not caused by 'synchronizers'. Biological rhythms must be taken into account in studies on potassium metabolism especially in connection with deficiencies of man.

Statistical correlations, however, do not fully satisfy our scientific curiosity. We want to know causal relations too. In the medical practice it is of vital importance to know the causes of a symptom, notwithstanding the fact, that a physician often cannot do more than fight against the symptoms because their causes are not known or are inaccessible to the medical means.
On the other hand drugs applied for curing one deficiency may cause other deficiencies. This is the case especially for drugs affecting the potassium metabolism. Mr. *Perlemuter* has reported a long list of such examples, a list which also gives a lot of information on the mode of action of the drugs.

Fortunately the animal and human body has a certain buffer capacity against external influences. But this capacity is limited and an overcharge induces insufficiences and deficiencies.
Mr. *Apfelbaum* has found this to be true for the K-protein enzyme-ratio in man and rat. This ratio is constant and is not changed by aging or undernutrition; a potassium deficiency always induces a negative nitrogen balance.
Mr. *Hartmans* too, explains the results of his experiments with cattle with the buffer capacity of the animal body: High daily doses of $CaCO_3$ increased the absorption of K, Na, Mn and PO_4, but the balance of K, Na and Cl was kept constant by an increased excretion of these with the urine.

Many physiological processes apparently are independent of each other. In the experiment of Mr. *Scott* for e.g., sheep and calves excreted excess acid or K infused into the rumen without competition between acid and K.

Finally we may approach the potassium metabolism in a fully abstract and theoretical way.
Physiological processes primarily are chemical reactions. Mr. *Evans* has summarized what is known about these up till now. In living organisms these reactions are controlled by an ordered arrangement in space and in time.
A definite spatial arrangement is linked to the solid state. Chemical reactions on solids are confined to the surface of the solid. Membranes are solids with a high specific surface.
Chemical reactions come to an apparent standstill — equilibrium — unless the reaction products are removed and new educts are adduced. Membranes are ideal means for separation; besides the big surface they offer short transport ways.
The selectivity itself may be linked to the molecular structure of the membrane, to specific carriers and to an active, energy consuming transport.
Mrs. *Koefoed-Johnson* has demonstrated the problem of selective separation by classic experiments with frog skin.

Electronmicroscopy has advanced morphology already to very fine structures. The next step will be to determine the arrangement of definite molecules in these structures and by combination of both chemical reactions and structures we will get a far better picture of what we call physiological processes.

4th Working Session

General Discussion

Chairman of the Session

M. *G. Drouineau,* Inspecteur Général de la Recherche Agronomique, Paris/France; Member of the Scientific Board of the International Potash Institute.

General Discussion

Chairman: G. DROUINEAU, Inspecteur Général de la Recherche Agronomique, I.N.R.A., Paris/France; Member of the Scientific Board of the International Potash Institute

Dr. *R. Scott Russell* (Wantage/England):

Although there is clear evidence that the absorption of ions by plant roots is dependent on concurrent metabolism, direct evidence on the nature of the mechanisms responsible for absorption in intact plants remains meagre in the majority of cases. A number of successive steps occur in the transfer of ions across plant roots; we have no reason to believe that they are related in a constant manner. Hitherto we have lacked the ability to examine each separately. Thus it is often difficult to distinguish between relationships which are casual from those which are causal. It is perhaps not unfair to suggest that we are sometimes in a rather similar position to someone who, attempting to study the effects of climate on activities in the island where I live, concluded that there was a causal relationship between the yield of wheat and the number of runs scored in cricket during May. As rain above the average in that month not only discourages cricketers but also can retard the development of the wheat crop some correlation between the two is possible — but implies nothing regarding mechanisms!

Those who investigate uptake mechanisms in animal tissues which show specialized absorption systems, or in coenocytes, have in general been able to conduct far more detailed and satisfying analyses. Yesterday Dr. *Koefoed-Johnsen* gave an excellent example of the detailed work on frog skin carried out in association with Professor Dr. *Ussing*. There have also been a number of references in the Colloquium to the successful investigations of ion pumps in coenocytes, for example, by *Dainty* and *MacRobbie*. It seems reasonable to imagine that the general nature of mechanisms in plant root systems may be broadly comparable to, though not necessarily identical with, those that have been demonstrated in these detailed studies. To sum up at the present stage of work on intact plants it seems prudent to have very clearly in mind the limitation of our ability to advance detailed interpretations of transport mechanisms.

Kinetic relationships observed between external concentration and absorption are an inadequate basis for identifying mechanisms; some of the reasons for this were discussed in the second session of this Colloquium.

I do not think it defeatist or pessimistic to acknowledge these limitations to our present understanding of uptake mechanisms in plants. During the course of this Colloquium references have been made to many types of new experimental approaches which should enable us to analyse the performance of plant roots in considerably greater detail in the future.

Dr. *V. Koefoed-Johnsen* (Copenhagen/Denmark):

As I did not take the time yesterday to describe how the distinction between active and passive processes can be made I would like to use this opportunity to give a brief summary of the methods used for analyzing transport processes. Some transport phenomena can be explained by means of physical forces like diffusion down a concentration gradient, distribution according to electrical potentials or movements caused by solvent drag. A certain number of transport processes cannot, however, be explained as a result of physical forces but involves participation of some energy-yielding chemical reactions. For that kind of process we use the term active transport. Generally active transport is defined as a process where the substance in question is moved against its electrochemical potential, in other words the substance gains free energy by the transfer process. This extra free energy is delivered by the metabolism of the cell. In no case of active transport though has the underlying molecular mechanism been fully clarified.

Calculation of the electrochemical potential for a given ion species requires knowledge of the extra- and intracellular concentrations of the ion in question and of the electrical potential difference across the membrane as measured by means of microelectrodes. A given ion will then be in equilibrium with respect to a given membrane when its electrochemical potential in the inside solution $\bar{\mu}_{(i)}$ equals its electrochemical potential in the outside solution $\bar{\mu}_{(o)}$, the electrochemical of the j'th ion being defined by $\bar{\mu}_{(i)} = RT \ln a_j + zF\psi + I$ where R,T, z, F have the conventional meaning, a_j denote chemical activity, ψ electrical potential, and I, a constant. Deviations from the equilibrium condition $\bar{\mu}_{(o)} = \bar{\mu}_{(i)}$ are indicative of active transport. The equilibrium analysis has certain limitations, the main one being that most biological systems are not in an equilibrium state, but change their composition constantly. Furthermore it might be quite erroneous to assume that the activity coefficients are the same outside and inside the cell and that the potential determined by the microelectrode is thermodynamically significant. Finally the unidirectional flux is a very involved function of the membrane structure as well. If, however, the flux ratio (i.e. the ratio between the simultaneously determined influx and efflux) is considered instead of the unidirectional flux this ratio can be shown to be quite independent of the membrane structure and exclusively determined by the ratio between the electrochemical activities in the bathing solutions: $M_{j(i)}/M_{j(o)} = \bar{a}_{j(o)}/\bar{a}_{j(i)}$ or disregarding the activity coefficients

$$M_{j(i)}/M_{j(o)} = c_{j(o)}/c_{j(i)}\, e^{\frac{zF}{RT}(\psi_1 - \psi_2)}$$

M = flux, a = electrochemical activity.

The fluxes can be measured by means of suitable tracers, and the electrochemical activities can be calculated from the inside and outside concentrations and the measured potential. Agreement between the two estimates of the flux ratio means that the ion in question moves passively.

Still a third factor might influence the passive movement of ions. If we are bealing with a porous membrane through which water forms a continuous phase the flow of solvent exerts a force on the diffusing particles so that molecules moving against the flow are slowed down while molecules moving in the direction of flow are speeded up. For a porous membrane with solvent flow the flux ratio equation must contain an additional term describing the solvent drag:

$$\ln (M_{j(i)}/M_{j(o)}) = \ln (c_{j(o)}/c_{j(i)}) + zF(\psi_1 - \psi_2)/RT + k\Delta w/D$$

where k is a constant characteristic for the membrane and independent of the solute as long as it can permeate the membrane. Δw is the volume rate of the flow of water, D the diffusion coefficient for the solute in question. The drag effect on an ion can be estimated by measuring the flux ratio for an uncharged molecule of the same size.
The drag effect is no doubt of importance for those biological systems where osmotic and hydrostatic pressures occur.
In summary: a given process can only be described as active transport by a process of elimination. However, where the electrochemical activities of the inside and outside solutions are identical (e.g. the short-circuited frog skin bathed with identical solutions) a net transfer of an ion can only be accounted for by active transport.

Dr. *L. Perlemuter* (Créteil/France):

Leakage of K out of cells, especially when inhibitors or drugs are applied:
1) Leakage of K^+ out of cells with accumulation in blood is a common problem in current medicine, especially in reanimation. In general this leakage is seen when there is a tendency to acidosis (best example is diabetic acidosis, but also other conditions like renal acidosis).
When this is realised there is at the renal level an exchange between K^+ and Na^+ but also with other ions. If kidney fails to eliminate this K^+ the blood will contain a high level of this ion and the toxicity on living organs will begin from a level of 6 meq/l. We admit that a vital risk is attained about at 7 meq/l. In general K^+ accumulation occurs especially in renal insufficiency.
2) For these reasons we can oppose leakage of potassium with urinary loss in connection with influx of Na^+. This condition is seen, when mineralocorticoids — i.e. in man aldosteron is applied or abnormally secreted.
There one can see a tremendous loss of potassium in urine as, in the same time, sodium is retained — (in a second time there will be an adaption as 'escape phenomenon' and the total amount of Na^+ in organism will not exceed a certain quantity).
But, any way, muscle biopsy can show that the content of muscle cell is changed, so that Na^+ has replaced K^+ in an important proportion. This phenomenon can be objectivated more easily for the physiologist by the measurement of Na^+/K^+ in saliva secretions, etc.
Hyperaldosteronism can be primary (adrenal disease secreting the hormone), secondary through stimulation by the renin angiotonin system or iatrogenic (corticotherapy).
This leakage of K^+ is essentially due to the hormone action on the cell mechanism of $Na^+ - K^+$ exchange. It can be suppressed by anti-aldosterone (Aldactone) that blocks the action of aldosterone acting on the DNA-RNA level.

Prof. Dr. *H. Marschner* (Berlin/Germany):

Leakage of K^+ out of cells, especially when inhibitors or drugs are applied:
Normally in a living cell influx of K^+ is much higher than the efflux. The term leakage is used when the efflux is higher than the influx. Leakage of K^+ (or other ions or small molecules) can be induced by using metabolic inhibitors like 2,4 DNP. Leakage of K^+ can also occur when the total amount of negative charges within the cell decreases e.g. by metabolizing organic anions or in connection with protein metabolism. Leakage of K^+ can

also occur, when several drugs or antibiotics like gramicidin, nonactin or mycostatin are applied. These substances presumably react with certain constituents of the membranes like lipids, glycolipids or proteins anc by this increase the membrane permeability and causes leakage of K^+. From our experiments we have evidence that mycostatin is quite different effective in inducing leakage of K^+ when applied to different plant species. This different effect of mycostatin may be caused due to differences in the membrane of the different plant species. Analysis of the membrane constituents of different plant species — especially the lipid fraction — in combination with experiments using antibiotics and studying their effect on K^+ leakage will give us further information about the properties and the transport of K^+ through the membranes.

Mr. *M. Brochart* (St. Génès-Champanelle/France):

I should like to make a point clearer mentioned by Prof. *Bach*. I did not find a correlation between K/Na on hair and fertility of cows. Indeed I find a positive correlation between K/Na in the food intake and K/Na in hair, which is understandable as we know that excretion of K and Na by sweat is influenced by the K and Na intake; as hair is able to fix K and Na excreted by sweat, we may understand the observed correlation. As concerns the role of a possible K/Na desequilibrium in food intake on cows fertility, the absence of correlation between K/Na in hair and the fertility of cows does not support the view supported by some authors on the negative direct effect of an excess of K intake on fertility.

Prof. *Y. Coïc* (Versailles/France):

Like many authors we demonstrated that the accumulation of organic acids is a direct effect of the metabolism of the nitrates *in situ*. In an experiment with tobacco we showed that the suppression of NO_3 in the nutrient solution stopped the accumulation of the organic acids in the leaves. In this same experiment we were able to show the variation of the velocity of the accumulation of the organic acids with time and consequently of the velocity of metabolism of the nitrates in the young, adult and old leaves. If the nitrates are metabolized in the roots, the electrostatic equilibrium is assured by a larger absorption of anions compared with that of cations, which provokes a 'physiological alcalinisation'; the latter varies considerably for the vegetal species, since it is a function of their power to metabolise the nitrates in the roots. In the soil this physiological alcalinisation reveals some importance since it is localised around the roots.

Answer to Dr. Kirkby: It is difficult to pass from the determination of the activity of the nitrate-reductase of the roots *in vitro* to the metabolism of the nitrates, that means to the transformation of the nitrates in amino acids and amides in the roots. Particularly if the activity of the nitrate — reductase *in vivo* is not the limiting factor in the chain of transformation of the nitrates in amino acids and amides the determination of its activity does not present an important contribution to the knowledge of the power of metabolism of the nitrates in the roots considered.

Dr. *W. Dijkshoorn* (Wageningen/The Netherlands)

The fact that gramineous plants have a carboxylate (C.A.) content, one-half the organic N does not necessarily mean that nitrate is reduced by the roots. In fact, analysis of bleeding sap in maize showed that nitrate plus the other anions of the nutrient salts move up completely balanced by metal cations. This means that maize generates caboxylates in amounts equivalent to the nitrate anions converted into organic N in the leaves. But maize retains only one half the carboxylates produced by the metabolism. Apparently the loss of carboxylates comes from the phloem. Transfer to the roots, subsequent decarboxylation and release of bicarbonate in the medium thus creating the external alkaline effect. Strictly speaking, the latter does not originate from differential uptake, but from release of carboxylates after their conversion to bicarbonate.

Dr. *A. Kylin* (Stockholm/Sweden): *Some Approaches to the Biochemistry of Carriers*

It has been said in the discussion here that there is little that can be done to reach an understanding of how carriers, or permeases as they may be called in another terminology, work in the transport of ions. Luckily, I do not think the situation is as bad as that. At least 3 experimental approaches can be mentioned, which give some information about the biochemical possibilities and which may, hopefully, be developed into a deeper understanding.

Pardee used *Escherichia coli* to investigate the possible role of proteins in specific permeases. The organism can be induced to transport sulphate, provided that it is grown with SO_4 in the medium. Upon fractionation of a homogenate of induced bacteria, a protein with specific binding capacity for sulphate can be isolated. Such a protein is not present in non-induced control cultures of *Escherichia*. It seems reasonable to think of this protein as part of a carrier system. Similar units were isolated also from other biological systems, where the technique was applicable (review by *Pardee*).

All plants must have membranes that are adapted to function in the habitat, where the plant grows or where we want it to grow. Therefore, plant breeders have, without knowing it, created a selection of materials which can be used for comparative biochemical studies, giving information on correlations between habitat and membrane composition. Since the different varieties can be cultivated in different controlled conditions, it is possible to ask questions both as regards genetically fixed and adaptative properties.

This approach was used by *Kuiper*. He had access to a material of rootstocks of 5 varieties of grapes with varying salt resistance, which varieties were provided by Dr. *L. Bernstein*, of the *U.S. Salinity Lab.* in Riverside, Calif. The lipids from the roots were extracted and analyzed. The results of the first investigation may be summerized thus:

Lipid	Rootstock	
	Least resistant (high chloride)	Most resistant (low chloride)
Monogalactose diglyceride	high	low
Phosphatidyl choline	low	high
Phosphatidyl ethanolamine	low	high

Kuiper then used the lipids isolated for experiments in vitro. Monogalactose diglyceride in an organic solvent facilitated the transport of chloride through the solvent, whereas phosphatidyl choline and phosphatidyl ethanolamine facilitated the exchange of sodium for potassium. Thus, there is a clear correlation between the amounts of specific lipids found, their function as ion-carriers through a lipophilic phase, and the characteristics of different varieties with regard to salt transport and salt resistance. One may recall that the function of membrane systems and the carriers contained in them may be determined not only by the proteins but also by the surfactant lipids *(Benson; Green* and *Tzagoloff).*

A third approach may be possible by analyses of $(Na^+ + K^+)$-activated ATPases or similar systems, which are regarded as the biochemical expression of transport of the related ions *(Skou; Fisher, Hansen* and *Hodges).*

This has been done by us *(Kylin, Kuiper* and *Hansson)* separating the lipids and their fatty acids in sugar beet and in a series of ATPases prepared from sugar beet roots. Special regard was given to the phosphatidyl choline and sulfolipid fractions, since it has been possible to reconstitute defatted plant ATPases by the use of these two lipids *(Kuiper).*

As a general feature, the sugar beet seedlings were surprisingly high in sulfolipid – in the roots 37% of the total lipid content occured in this fraction. In preparing a root homogenate with properties as an unspecific ATPase, almost all the phosphatidyl choline and much of the phosphatidyl ethanolamine but only 5 per cent of the total sulfolipid followed the fraction. Calling forward the $(Na^+ + K^+)$ specificity by a treatment with deoxycholate for 1 hour, reduced the lipid contents by a factor of 2. Unproportionally high amounts were lost from the zwitterionic phosphatidyl choline and the related phosphatidyl ethanolamine, whereas the acidic sulfolipid and phosphatidyl inositol were, proportionwise, well retained. Also as regards the contents of long-chain (around C_{26}) fatty acids, a fractionation was evident:

Material	C_{26} as percent of total fatty acids in	
	phosphatidyl choline	sulfolipid
Whole roots	13	2
Control ATPase	16	8
$(Na^+ + K^+)$-activated ATPase	4	21
Dead ATPase	4	7

To the extent that these changes are reflected in the properties of the preparations, they should mean that a higher proportion of negative surface charges are exposed when the $(Na^+ + K^+)$-specificity is induced. This could make sense chemically. The negative charges might be a pre-requisite to bind the highly mobile monovalent cations; which at the same time, could be necessary to neutralize the negativity and bring the swollen structures back into a conformation, where they can act as ATPases. It was later shown by particle electrophoresis that negative charges are, as a matter of fact, revealed upon inducing the $(NA^+ + K^+)$-specifity of the ATPases (*Karlsson, Tribukait* and *Kylin*).

In passing, it may be mentioned that more than 50% of the fatty acid complement of the cerebroside and of the phosphatidyl choline in the stalks was found as as $[C_{26} + C_{28}]$. In the leaves 20 to 25% of the sulfolipid and of the phosphatidyl inositol were conjugates of $[C_{26} + C_{28}]$. Reasonably, such a composition is somehow related to function, although no interpretation can be offered for the moment.

Summarizing, several approaches are available to attack the problem of how carriers are built up and how they work, and higher plants may offer some of the best experimental materials available.

References

1. *Benson, A.A.:* Plant Membrane Lipids. Ann. Rev. Pl. Physiol *15*, 1–16 (1964).
2. *Fisher, J.D., Hansen, D.* and *Hodges, T.K.:* Correlation between ion fluxes and ion-stimulated adenosine triphosphatase activity of plant roots. Pl. Physiol *46*, 812–814 (1970).
3. *Green, D.E* and *Tzagoloff, A.:* Role of lipids in the structure and function of biological membranes. J. Lipid Res. *7*, 587–602 (1966).
4. *Karlson, J., Tribukait, B.*, and *Kylin, A.:* Electrical mobilities of fragments of sugar beet root membranes with properties as $(Na^+ + K^+)$-activated adenosine triphosphatases. Proc. 1st Europ. Biophys. Congr. Vienna *III*, 75–79 (1971).
5. *Kuiper, P.J.:* Lipids in grape roots in relation to chloride transport. Pl. Physiol. *43*, 1367–71 (1968 a).
6. *Kuiper, P.J.C.:* Ion transport characteristics of grape root lipids in relation to chloride transport. Pl. Physiol. *43*, 1372–74 (1968 b).
7. *Kuiper, P.J.C.:* Temperature response of adenosine triphosphatase of bean roots as related to cold sensivity and lipid composition of the roots and the lipid requirement of the adenosine triphosphatase. Physiol. Plant. *26*, 200–205 (1972).
8. *Kylin, A., Kuiper, P.J.C.* and *Hansson, G.:* Lipids from sugar beet in relation to the preparation and properties of (sodium + potassium)-activated adenosine triphosphatases. Physiol. Plant *26*, 271–278 (1972).
9. *Pardee, A.B.:* Membrane transport proteins. Science *162*, 632–637 (1968).
10. *Skou, J.C.:* Enzymatic aspects of linked transport of Na^+ and K^+ through the cell membrane. Progr. Biophys. Mol. Biol. *14*, 131–166 (1964).

Closing Address

Monsieur le Prof. Jansson,
Messieurs les membres du Colloque scientifique,
Messieurs et Mesdames les conférenciers,
Mesdames et Messieurs,

Au moment de prononcer la clôture officielle de ce Colloque d'Uppsala, c'est avec une profonde satisfaction que je me sens pressé d'adresser mes remerciements aux personnalités qui en ont assuré le succès.

Nous avons été très sensible à la présence de Monsieur le Ministre *Ingemund Bengtsson* lors de la séance d'ouverture. Son propos a témoigné de l'intérêt qu'il a bien voulu porter à nos travaux. Nous renouvelons ici à *Monsieur le Ministre de l'Agriculture de Suède* l'expression de notre profonde reconnaissance.

Cette gratitude va aussi à Monsieur *Hjelm*, Recteur de l'Université Agronomique d'Uppsala, qui a bien voulu mettre à notre disposition certains moyens de sa Haute Ecole, où l'accueil, qui nous fut réservé, constituera l'un des beaux souvenirs de notre séjour dans ce pays.

Je dois naturellement un merci tout particulier à Monsieur le Prof. *Jansson* — non seulement pour avoir présidé ce colloque avec une rare compétence — mais aussi pour l'avoir si bien préparé aux côtés de Monsieur *Dam Kofoed*, de Monsieur le Prof. *Mengel* et de Monsieur le Directeur *de Tarragon*.

Je ne saurais enfin terminer ce message sans dire à Monsieur le Prof. *Jelenic*, à Monsieur le Prof. *Mengel*, à Monsieur le Prof. *Bach* et à Monsieur l'Inspecteur Général *Drouineau* combien nous leur savons gré d'avoir assuré la coordination des séances et la présidence de la discussion finale. Vous ne m'en voudrez pas de ne pas citer nommément les noms de tous nos conférenciers ou auteurs de communications. Votre participation, Messieurs, a contribué à la tenue scientifique d'un débat que nous avions l'espoir de porter à ce niveau. Premier colloque d'un nouveau cycle d'études, il importait que la rencontre d'Uppsala assurât cet apport de valeur, qui fait bien augurer de la suite de nos travaux.

J'ajoute à mes remerciements ceux que je dois à nos Directeurs de l'Institut Messieurs *von Peter* et *Künzli*, ainsi qu'à leur personnel.

Il ne m'appartient pas de revenir ici sur les conclusions de nature scientifique qui ont fait l'objet de la discussion finale.

Je me borne à retenir deux faits qui me paraissent avoir marqué le colloque.

Le premier est de portée purement formelle, mais il a son importance. Les conférenciers, dont les travaux vous avaient été remis à l'avance, se sont tenus d'assez près à leurs textes, permettant ainsi une assimilation plus facile des idées exposées et laissant une place

suffisante aux discussions si nécessaires pour compléter les données des conférences et fouiller les problèmes de manière approfondie.
Le second fait que je veux relever est celui qui constituait l'innovation de ce colloque, à savoir «l'échange interdisciplinaire de connaissances scientifiques». L'expérience devait être tentée. Elle le fut avec un plein succès. Ce n'est pas sans appréhensions que nous l'avons entreprise. Et cette innovation introduite dans la structure générale du Colloque ouvre dorénavant devant nous de larges perspectives, un avenir prometteur.
On pouvait se demander en effet si les études envisagées pour de prochains colloques n'allaient pas insérer notre Institut dans un couloir trop étroit de préoccupations. Encore ce risque était-il relatif au vu de nos intentions, puisque nous aborderons l'an prochain le problème de *«K dans le sol»*, en 73 les *«aspects spécifiques de la dynamique du K pour les cultures tropicales»* et au Congrès de 1974 le thème général du *«Rôle spécifique de K. sur les sols et les plantes et ses effets sur la production végétale»*. Il y a là matière à de solides études qui répondent aux préoccupations de tous les chercheurs engagés dans ce domaine particulier de la discipline scientifique.
Cependant, l'expérience qui vient d'être faite a été réalisée sous le signe de l'interdépendance des études dont on risque de méconnaître les implications en physiologie humaine, en thérapeutique, dans des secteurs où tous les éléments de la connaissance ont leur place et jouent leur rôle. Il n'est pas excessif de dire que les chercheurs travaillant en physiologie humaine ont été nos hôtes d'honneur. Grâce à leur contribution, le colloque d'Uppsala ne manquera pas d'avoir un certain retentissement. Il sera l'expression d'une mise en garde contre le risque du cloisonnement qui marque notre société contemporaine à l'heure de l'explosion des techniques nouvelles, alors qu'apparaissent dans toute leur ampleur les conséquences de la recherche appliquée. Paradoxalement, ce compartimentage des hommes et de leurs activités se fait sentir dans une partie du monde rétrécie et surpeuplée. Ce phénomène ne manque pas d'être inquiétant en ce sens qu'il conduit à l'élaboration d'une société anonyme et déshumanisée. S'il y a là matière à réflexion pour les hommes préoccupés de l'évolution sociologique du monde, l'idée de l'échange interdisciplinaire de connaissances scientifiques éveille un espoir dès lors que les hommes se rendent compte qu'ils ne peuvent ni s'ignorer, ni garder chacun pour soi les parcelles de cette connaissance dont le partage est générateur de sécurité et de progrès. Votre réflexion de Skokloster marque donc un tournant dans les activités de l'Institut, dont les préoccupations iront s'élargissant et affirmeront toujours davantage une raison d'être et une conviction que de grands pas en avant sont encore à faire dans l'avenir.
Je dis une fois de plus à nos amis de Suède l'excellent souvenir que nous emporterons d'un lieu de travail qui ne pouvait se prêter mieux aux buts que nous recherchions et d'un accueil dont la simplicité directe et amicale nous est allée droit au cœur. En vous renouvelant mes vœux pour le succès de vos activités et votre bonheur personnel, je vous souhaite un bon retour dans les pays où vous œuvrez, où vous ne cessez de contribuer à leur prospérité morale et matérielle.
Merci encore à tous! Le Colloque d'Uppsala est terminé.

P. Chaudet
Ancien Président de
la Confédération Helvétique;
Président de l'Institut
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