RADIOISOTOPES IN SCIENTIFIC RESEARCH

VOL IV

RADIOISOTOPES IN SCIENTIFIC RESEARCH

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VOLUME IV

Research with Radioisotopes in

PLANT BIOLOGY AND SOME GENERAL PROBLEMS

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The Transport of Bivalent Cations into the Yeast Cell in relation to Potassium and Phosphate Uptake

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Abstract

Studies with Ca^{45}, Sr^{90}, Mn^{54} and P^{32} indicate that bivalent cations, although they are reversably bound by the surface of the cell are not absorbed into the cytoplasm unless there is a concomitant uptake of phosphate, associated with the metabolism of sugars. In contrast to the surface binding of cations which is relatively non-specific the uptake with phosphate is highly specific for Mg^{++} and Mn^{++}. However, other bivalent cations although only absorbed slowly can competitively inhibit the uptake of Mg^{++} and Mn^{++}. Studies with Mn^{54} reveal that once absorbed, the Mn^{++} is no longer exchangeable. The rate of uptake follows a saturation type of kinetics, the maximal rate being conditioned by the amount of phosphate absorbed. All conditions which influence phosphate uptake, also influence the cation uptake. Inhibition of both uptakes is produced by dinitrophenol, azide or arsenate. Marked stimulation is produced by potassium. When K^+, Mn^{++}, phosphate and glucose are added simultaneously, the sequence of uptakes is K^+, PO_4 and Mn^{++}. The system behaves as though a carrier for Mn^{++} were synthesized during phosphate uptake, with the synthesis stimulated by potassium.

In recent years, with radioactive isotopes generally available, a great deal of new information has accumulated concerning metabolism of electrolytes, particularly sodium, potassium and phosphate, by cells. Isotope techniques have allowed the determination of the exchangeability of cellular ions as well as the rates of inward and outward movement. In many cases, a metabolism-dependent active transport of ions has been implicated as an important factor in the passage of these ions across the cell membrane.

Although useful isotopes of many of the bivalent cations have also been available (Mg^{++} is a notable exception), they have, in general, been less intensively investigated. A review (1) of studies carried out with plant cells, particularly roots, indicated the existence of two distinct processes. Firstly, the bivalent cations are bound on the surfaces of the cells in an exchangeable form. Secondly, they are transported into an "inner space", becoming non-exchangeable. In yeast cells a similar situation obtains, in the case of Mn^{++}, with surface bound cations being freely exchangeable, and those transported into the cell being non-exchangeable (2,3). In muscle, studied with Ca^{45} both the surface bound, and intracellular Ca^{++} are exchangeable but at different rates. The Ca^{++} is apparently actively transported out of the cell (4,5).

The binding of the bivalent cations to surface of cells has been demonstrated in a variety of cell types including plant leaves (6), plant roots (1), bacteria (7), yeast (8,9), spores (10), sea urchin eggs (11), and muscle (4). In the case of yeast, the binding sites have been characterized in some detail (8,9). On the other hand, little is known concerning the mechanism by which these ions pass into the cytoplasm. Recent studies with yeast cells (12,13), which will be reviewed here indicate that a highly specific transport mechanism is involved, which is linked directly to phosphate transport and indirectly to potassium transport, but is not dependent on the ion binding properties of the cell surface. In most of the studies, isotope techniques were invaluable, not only because they provided specific means of quantitating the uptakes of cations difficult to measure by chemical procedures, but also because they provided a means for measuring the exchangeability of given cellular action. Isotopes used in the studies included Mn^{54}, Ca^{45}, Sr^{90}, P^{32}, U^{233}, and K^{42}.

METHODS

Fresh baker's yeast (standard brands) was used in all experiments. The cells were thoroughly washed by suspending in water and centrifuging. The speed of centrifugation was low so that colloidal material and cellular debris was discarded. After aeration for several hours, the isotopes, electrolytes, and substrates were added. At appropriate times, the cells were separated by high speed centrifugation (10,000 x g) and the counts and analyses carried out, in most cases, on the supernatant solutions.

The P^{32}, Sr^{90}, Ca^{45}, and K^{42} were counted with a thin window Geiger tube, the Mn^{54} with a deep—well scintillation counter and the U^{233} with an alpha counter after electro—plating on silver foil (14).

Chemical analyses were carried out as follows:
phosphate: method of Fiske and Subbarrow (15), modified by the addition of 15% ethyl alcohol before color development
sodium and potassium: flame photometer
Mg: precipitation as magnesium ammonium phosphate in the presence of an excess of ammonia and phosphate, with 20% ethyl alcohol. The washed precipitate is analysed for phosphate (present in a 1/1 ratio to Mg).
Ca: versene—titration with eriochrome black T.(16).

RESULTS

Binding of Cations by the Cell Surface

The first studies on cation binding by the cell surface of yeast were carried out with UO_2^{++} (8). The interaction between the ion and the cell was characterized in terms of a simple mass law reaction:

$$U + Y \rightleftharpoons UY$$

in which U represents the cation, Y a fixed anion of the cell surface, and UY the complex between the two. The number of Y groups, based on the saturation curve for U—binding, was approximately 1 mM per kg of cells, which can be compared with a K-content of 150 mM per kg of cells and a Mg—content of 20 mM per kg of cells. In other words, the cell surface binding sites can account for only a small fraction of the cation content of the cell. The chemical properties of the Y groups, based on their behaviour with respect to UO_2^{++}—binding, resemble those of polyphosphate compounds such as nucleic acids or phosphate polymers (17).

An additional species of UO_2^{++}—binding sites was demonstrated at higher concentrations of UO_2^{++} (18). The binding of UO_2^{++} to the "phosphate" sites was associated with inhibition of sugar uptake, and the binding of UO_2^{++} to the second type of binding site, presumably carboxyl groups of proteins, was associated with the inhibition of the invertase activity of the cell surface. The existence of two species of cation binding sites was most clearly demonstrated by studies with Mn^{++} (using Mn^{54}) (9). An appropriate mass—law plot of the binding data can be represented by two straight lines, the steeper slope representing the "phosphate" sites and the shallow slope, the "carboxyl" sites.

All cations tested, including monovalent cations, such as Na^+ and K^+ are reversably bound by the cell surface sites, but the stabilities of the complexes formed vary considerably: UO_2^{++} forming an especially stable complex; other bivalent cations forming stable complexes; and monovalent cations forming weak complexes.

The Transport of Bivalent Cations into the Cell

The bivalent cation content of the baker's yeast, used in the present experiment, is approximately 23 mM per kg of cells; with individual values: Mg, 20 mM; Ca, 2 mM; Mn, 0.2 mM; and others present in trace amounts. Studies with Ca^{45} and with Mn^{54} indicate that with the exception of the small fraction associated with the surface binding sites, the cellular bivalent cations are not readily exchangeable, either in resting or actively metabolizing cells (2). Nevertheless, the cells are able, under certain conditions, to absorb large quantities of bivalent cations. For example, Schmidt *et al.* (19), studying the uptake of phosphate during metabolism of sugars, found that large quantities of K^+ and Mg^{++}

were also absorbed.

The absorption of bivalent cations has been recently investigated in some detail (12,13) using Mn^{54}. In the studies, the two processes, surface binding and absorption, could be readily separated on the basis of exchangeability; the Mn^{++} bound to the cell surface being completely exchangeable, and that absorbed being nonexchangeable. Furthermore, the binding occurred whether substrate was present or not, whereas, the absorption only occurred when both a substrate *and* phosphate were present, with a marked stimulation by potassium. For example in Table 1, with phosphate only, glucose only, or glucose and K^+, the uptake of Mn^{++} ceased in less than three minutes, with all of it exchangeable. With glucose and phosphate, or glucose, phosphate, and K^+ the uptake continued to completion and the Mn^{++} was no longer exchangeable.

Table 1. Binding and Uptake of Mn^{54} as influenced by various factors

	Mn—Uptake in %	
	2 min.	30 min.
Control	25	24
+ K^+	23	23
+ PO_4	23	27
+ K + PO_4	24	22
+ glucose	25	24
+ glucose + PO_4	25	56
+ glucose + K + PO_4	33	100

Concentrations were as follows: Mn, 7.5×10^{-4} M/l, K, 2×10^{-3} M/l ; phosphate, 2×10^{-3} M/l; glucose, 0.1 M/l. The pH was 4.5 and the yeast concentration, 100 mg/ml.

The Relationship of Mn^{++} Uptake to Phosphate Uptake

Mn^{++} is only absorbed if phosphate is also absorbed, but the two ions are not necessarily taken up simultaneously. For example, the time sequence in Fig. 1. indicates that cells pretreated with glucose and K^+, take up Mn^{++} and phosphate at the same rate However, if K^+, Mn^{++}, and phosphate are added simultaneously, the K^+ is taken up very rapidly, but the Mn^{++} and phosphate are taken up only after a delay period of about 15 - 20 minutes. Furthermore, the Mn^{++} uptake lags behing that of the phosphate (12) The uptakes of the three ions can be separated in time. Cells pretreated an hour previously with K^+ and glucose, then washed, will absorb phosphate rapidly (with glucose added) with no further additions of K^+. If the cells are again washed and then suspended in Mn^{++} (plus glucose) with no further addition of phosphate or K^+, the Mn^{++} is rapidly absorbed. The ability of cells pretreated with K^+ and phosphate to absorb Mn^{++} disappears on standing. The half time for the decay process is 30 hours at 4 °C, 3 hours at 25 °C, and 1½ hours at 25 °C, if glucose is added (13)

The dependence of Mn^{++} uptake on phosphate uptake has also been demonstrated by the use of inhibitors of the latter process, such as dinitrophenol, acetate, redox dyes, and arsenate. In each case, the inhibitor concentrations are sufficiently low that the metabolism per se is little affected. In each case the extent of inhibition of phosphate uptake and of Mn^{++} are equal (13).

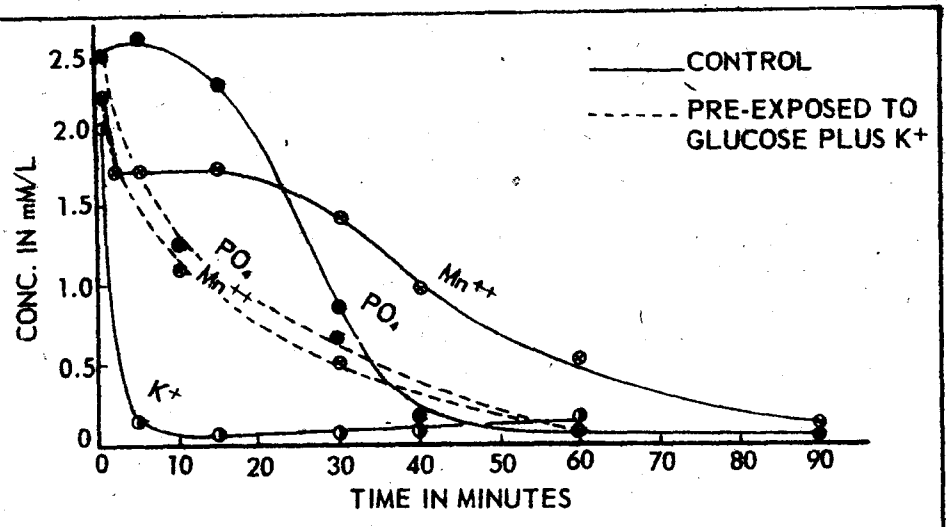

Fig. 1. The time course of the disappearance of K^+, Mn^{++}, and $H_2PO_4^-$ from the medium.

The yeast concentration was 100 mg/ml; pH, 4.5; temperature, 25° C; glucose, O.1 M. The dotted lines represent an experiment in which the cells were treated with glucose and K^+ an hour prior to zero time.

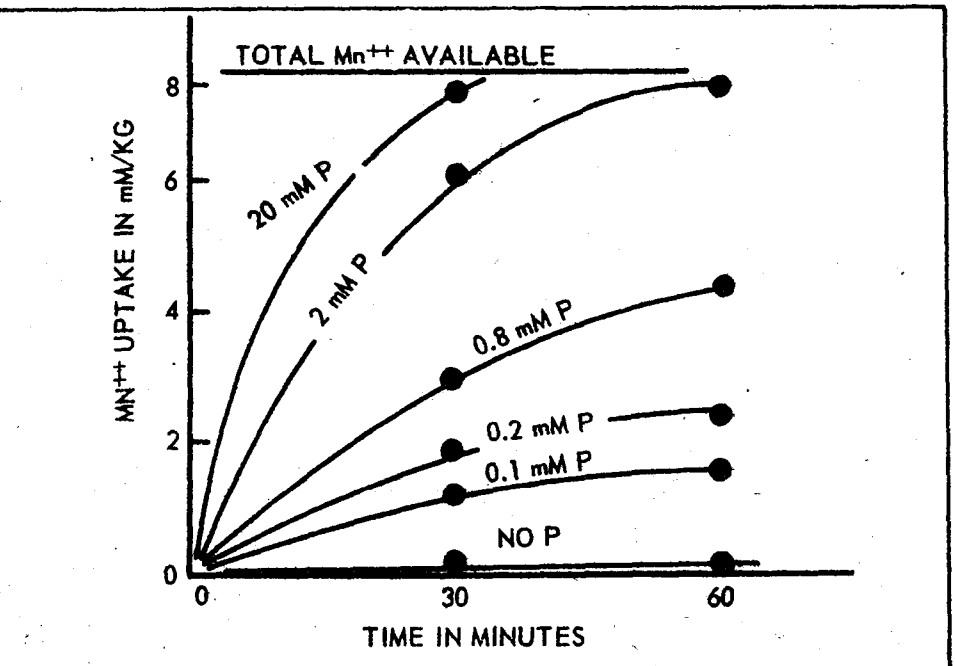

Fig. 2. The influence of phosphate concentration on Mn^{++} uptake.

The yeast concentration was 100 mg/ml; pH, 5.0; temperature, 25° C; glucose, O.1 M; and Mn^{++}, 7.5×10^{-4} M. The cells were pretreated with .01 M K^+ and glucose.

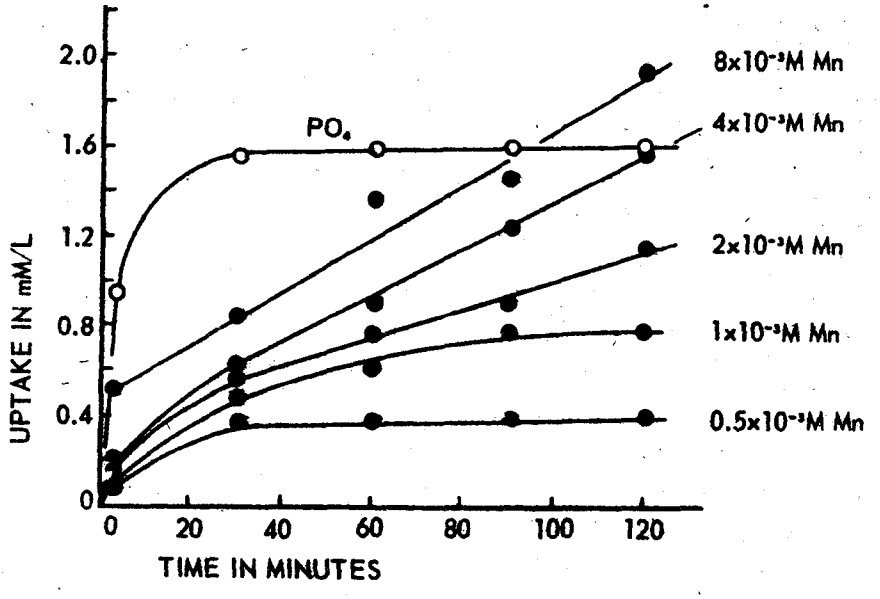

Fig. 3. The influence of Mn^{++} concentration on Mn^{++} uptake.

The yeast concentration was 100 mg/ml; pH, 5.0; temperature, 25° C; glucose, 0.1 M; and phosphate, 2 x 10^{-3} M. The cells were pretreated with 0.01 MK^{+} plus glucose.

Some of the quantitative interrelations between phosphate and Mn^{++} are shown in Figs. 2 and 3. The rate of Mn^{++} uptake increases with increasing concentrations of either Mn^{++} or of phosphate. However, the maximal amount of Mn^{++} uptake is conditions primarily by the amount of phosphate absorbed. For example, in Fig. 3, the maximal Mn^{++} uptake was in no case greater by a factor of 1.5 than the phosphate uptake, regardless of the phosphate concentration used. In all other experiments, in which an excess of Mn^{++} was used, the ratio of uptakes was approximately 1.0 with a range from 0.7 to 1.6 (12).

The dependence of Mn^{++} uptake on phosphate uptake, even though the two processes are separated in time, suggests that during the uptake of phosphate, a portion of it is synthesized into a Mn^{++}—carrier, a material which allows the cell to absorb Mn^{++}. The "carrier" decays on standing at a fairly rapid rate, with the rate increased during active metabolism. It is labile material coupled to metabolic reactions.

The Nature of Phosphate and Potassium Uptake in Yeast

Many of the properties of Mn^{++} uptake are referable to the properties of the phosphate uptake on which it is dependent. The latter process has been investigated in a variety of cells. Earlier studies with micro-organisms were reviewed by Spiegelman (20). In general, micro-organisms absorb phosphate by a metabolism-dependent transport system. In yeast, the phosphate, once absorbed, is converted primarily to metaphosphate (21,22), with little increase in the orthophosphate concentration of the cell (23).

Recent studies (23) indicate that phosphate is transported into the yeast cell against a 100/1 gradient, with energy supplied by glycolytic, rather than by respiratory, pathways. Although the transport follows a Michaelis-Menten kinetics, suggesting a reversable combination of phosphate in the transport system, no exchanges of phosphate occur between the cell and the medium. In other words, the transport is essentially a one way, inward movement of phosphate (just as in the case of Mn^{++}).

The action of potassium on Mn^{++} uptake is also referable, in large measure, to the effects of potassium on phosphate uptake (16). The uptake of phosphate is markedly dependent on potassium (19). However, the linkage between K^+ and phosphate is not a direct one. For example, during active metabolism, even in the absence of phosphate, potassium is taken up rapidly in exchange for H^+ ion, by a process that has been extensively investigated (2,24). The cytoplasm becomes more alkaline, with the absorbed K^+ balanced by HCO_3^- and by metabolic organic anions. The yeast cell can thus tolerate a surplus of fixed base. On the other hand, in the absence of potassium, the cell can take up a limited quantity of phosphate, the cytoplasm becoming more acid. Only a small deficit of fixed base can be tolerated. With both K^+ and phosphate present, the amount of phosphate absorbed is much greater. However, the stimulating action of K^+ is not related to any direct link between K^+ uptake and phosphate uptake, or to a direct stimulating effect of the extracellular K^+ on the phosphate transport system. In fact, it can be seen in Fig. 1. that all of the K^+ may be absorbed before phosphate uptake commences. In other experiments, pre-exposure of the cells to K^+ and glucose an hour earlier leads to an increased phosphate uptake. K^+ increases the phosphate uptake primarily because it provides the fixed base to balance the increased anion content.

The action of K^+ on Mn^{++} uptake can also be explained on the same basis. Fig. 3, indicates that low concentrations of K^+ stimulate the uptake of Mn^{++}, with a maximal effect at 4×10^{-3} M, but that higher concentrations of K^+ inhibit (12). Yet other studies indicate that the uptake of phosphate is progressively increased with increasing concentrations of K^+ (23). The difference in behaviour of K^+ toward phosphate and Mn^{++} is a reflection of the total electrolyte balance of the cell. Low concentrations of K^+ stimulate phosphate uptake which in turn provides the "carrier" for Mn^{++} uptake. High concentrations of K^+ stimulate phosphate uptake, but also "load" the cell with a surplus of fixed base, tending to prevent the uptake of other cations such as Mn^{++}.

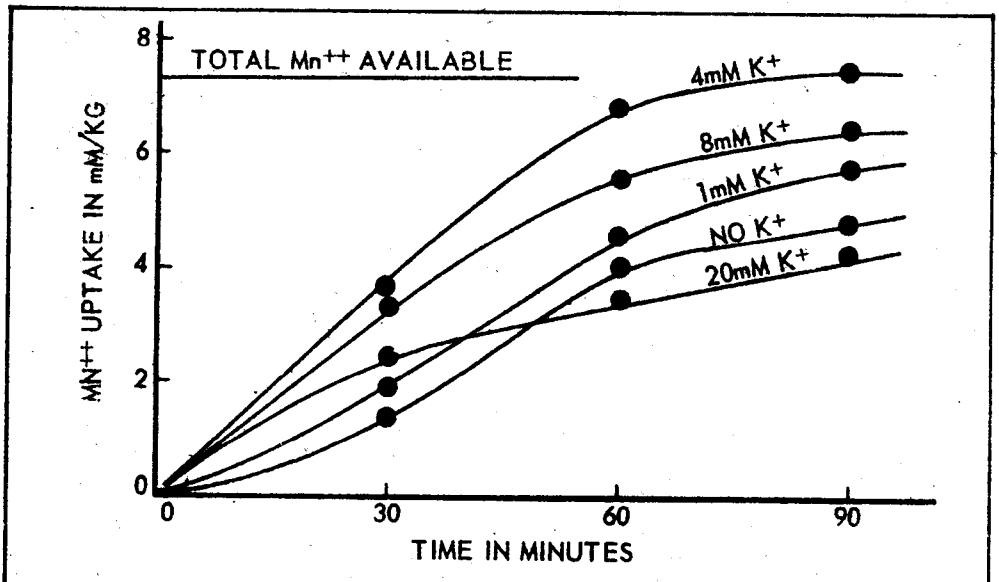

Fig. 4. The influence of K^+ concentration on Mn^{++} uptake.

The yeast concentration was 100 mg/ml; pH, 5.0; temperature, 25° C; glucose, 0.1 M; Mn^{++} and phosphate, 7.5×10^{-4} M.

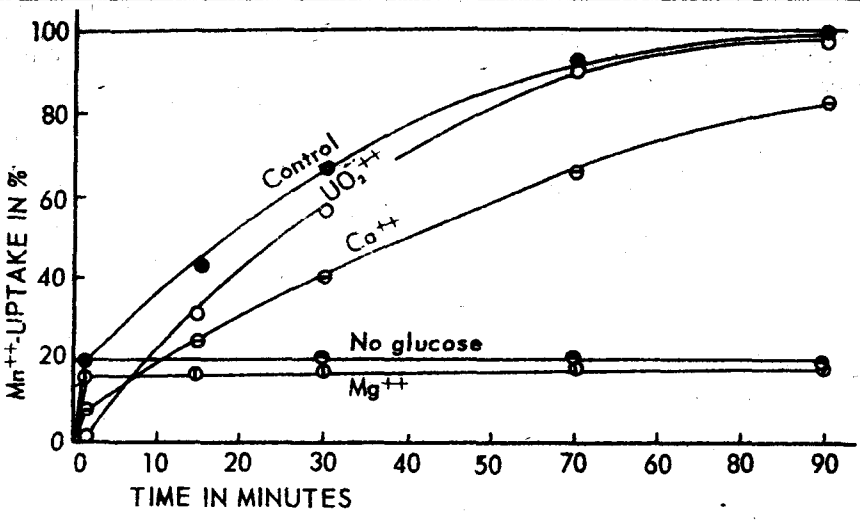

Fig. 5. The influence of UO_2^{++}, Ca^{++}, and Mg^{++} on Mn^{++} uptake.

The yeast concentration was 100 mg/ml; pH, 5.0; temperature, 25° C; glucose, 0.1 M; Mn^{++}, 1×10^{-3} M; UO_2^{++} $\times 10^{-3}$ M; Ca^{++}, 1×10^{-3} M; and Mg^{++}, 3×10^{-4} M.

Mn^{++}—Absorption versus Surface Binding

It has often been suggested that the first step in cation transport may involve adsorption or binding to the cell surface. In the case of yeast, surface binding has been demonstrated for both mono— and bivalent cations (9). Previous studies with K^+ indicate that binding by the cell surface is not a prerequisite for K^+ transport (25). Experiments involving the competitive action of a series of bivalent cations indicate that the surface binding of Mn^{++} is not a prerequisite for Mn^{++} uptake (12). The experiments were carried out with UO_2^{++}, Ca^{++}, and Mg^{++} as competing ions (Fig. 5). In the control (no competing ion), there was a rapid uptake of 20% of the Mn^{++}, which occurred with or without glucose (representing surface binding), followed by a slower continued uptake to completion, representing absorption into the cell. In the presence of UO_2^{++}, the surface binding of Mn^{++} was largely abolished, yet the uptake continued at an undiminished rate. UO_2^{++} has a marked affinity for the surface binding sites, but not for the Mn^{++}—transport system. In the presence of Ca^{++}, proportionate inhibitions were obtained on the surface binding and on the Mn^{++}—transport. However, with Mg^{++}, the Mn^{++}—transport was completely inhibited with only a small effect on Mn^{++} binding. Thus it is concluded that surface binding, at least to the sites accessible to these ions, is not a prerequisite to ion transport.

Specificity Pattern

The ion transport system under discussion is highly specific, with Mg^{++} preferred over Mn^{++} by a factor of at least 10 : 1, with only a small affinity for Ca^{++} and Sr^{++}, and almost none for UO_2^{++} etc. Thus the system studied with Mn^{++} is primarily a Mg^{++}—transporting system (12).

CONCLUSION AND SUMMARY

The interaction of bivalent cations and yeast cells involves two independent phenomena:

(a) the binding of the cations by fixed anionic groups of the cell surface, and (b), the specific transport of certain cations into the cytoplasm of the cell. The first process, the binding, is a strictly chemical phenomenon similar to that which would take place between cations in solution and fixed negative charges of any solid phase in contact with the solution. The bound ions are completely exchangeable, obeying appropriate derivations of the mass law. In yeast, at least two chemical species of binding sites are involved, each with a characteristic binding affinity for cations. Although the binding affinities for different cations cover a wide range, little discrimination between physiological important cations, such as Mg^{++}, Ca^{++}, Sr^{++}, and Mn^{++}, is evident. The surface binding of cations is not influenced by the metabolic state of the cell, nor is any relationship to electrolyte metabolism apparent. However, when certain heavy metal cations are bound, the ability of the cell to absorb sugars is impaired. (20).

In contrast, the absorption process is highly specific for Mg^{++} and Mn^{++} in comparison to Ca^{++} and Sr^{++}; it is directly dependent on cell metabolism, and on the uptake of phosphate and indirectly dependent on the uptake of K^{+}. The dependence on phosphate is apparently related to the synthesis of a phosphorylated "carrier" system, during phosphate absorption. For this reason, cells pre-exposed to phosphate (and substrate) can later take up Mn^{++} with no phosphate present. However, the "carrier" decays rapidly, especially in metabolically active cells. The exact nature of the "carrier" is unknown.

The relationship of K^{+} to Mn^{++} or Mg^{++} absorption is more complex, depending on the relative amounts of K^{+} and phosphate used. K^{+} stimulates phosphate uptake, thereby increasing the synthesis of the "carrier", and in turn, increasing the uptake of Mg^{++} or Mn However, this action is observed only if the amount of K^{+} absorbed is not greater than the amount of phosphate absorbed. On the other hand, if K^{+} is absorbed in greater amounts than the phosphate, an inhibition of Mg^{++} or Mn^{++} uptake results. The latter action is apparently associated with the inability of a cell with a surplus of fixed base (K^{+}) to absorb more cations.

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RESUME

Des études effectuées à l'aide de Ca^{45}, Sr^{90}, Mn^{54} et P^{32} montrent que les cations bivalents, bien qu'ils soient liés de façon réversible à la surface de la cellule ne sont pas absorbés par le cytoplasme à moins qu'il y ait incorporation concomitante de phosphate, associée avec le métabolisme des sucres. Par opposition avec la liaison de surface des cations qui est relativement non-spécifique, l'absorption avec le phosphate, de Mg^{++} et Mn^{++}, est très spécifique. Cependant d'autres cations bivalents bien qu'ils ne soient eux

mêmes absorbés que lentement peuvent par leur présence arrêter completement l'absorption de Mg^{++} et Mn^{++}. Les études avec Mn^{54} montrent qu'une fois absorbé le Mn^{++} ne peut plus être échangé. La vitesse d'absorption obéit à une loi cinétique caracterisée par une saturation, la vitesse maximum étant conditionnée par la quantité de phosphate absorbé. Toutes les conditions qui influencent l'absorption du phosphate, ont aussi une influence sur l'absorption du cation. L'arrêt des deux absorptions est produit par le dinitrophénol, un azide ou un arsénate . Le potassium au contraire stimule fortement l'absorption. Lorsque les phosphates de Mn^{++} et K^{+} et le glucose sont ajoutés simultanément l'ordre d'absorption est: K^{+}, PO_4, Mn^{++}: Le système se comporte comme si un entraineur de Mn^{++} s'était formé pendant l'absorption du phosphate, le potassium favorisant cette formation.

RESUMEN

Los estudios llevados a cabo con Ca^{45}, Se^{80}, Mn^{54} y P^{32} indican que los cationes bivalentes si bien son reversiblemente fijados por la superficie de la célula (Rothstein y Hayes, Arch. Biochem. 63, 87, 1956), no son absorbidos por el citoplasma, a menos que tenga lugar una captación concomitante de fosfato, asociada con el metabolismo de los azúcares. Contrariamente a la fijación superficial de cationes, que es bastante inespecífica, la captación con fosfato es altamente específica para el Mg^{++} y el Mn^{++}. Sin embargo, otros cationes bivalentes, aunque sólo son absorbidos con lentitud, pueden inhibir competitivamente la captación de Mg^{++} y de Mn^{++}. Los estudios realizados con Mn^{54} revelan que el Mn^{++} deja de ser intercambiable una vez absorbido. La velocidad de captación obedece a una cinética de tipo de saturación; la velocidad máxima es función de la cantidad de fosfato absorbida. Todos los factores que influyen sobre la captación de fosfato influyen asimismo sobre la captación del catión. Ambas captaciones son inhibidas por el dinitrofenol, la azida y el arsenato. El potasio produce un marcado estímulo. Al añadir simultáneamente K^{+}, Mn^{++}, fosfato y glucosa, la captación se verifica en el siguiente orden: K^{+}, PO_4 y Mn^{++}. El sistema se comporta como si durante la captación de fosfato se sintetizara un portador para el Mn^{++}, siendo esta síntesis estimulada por el potasio.

DISCUSSION

R.J. HELDER (Netherlands): You have told us that the effects of potassium and phosphate on Mn^{++} absorption are most probably due to the synthesis of a Mn^{++}-carrier. This carrier, however, disappears on standing. This decay of a carrier is of particular interest to me. In experiments on rubidium absorption by barley roots I found that absorption capacity can be reduced by pretreating the material with various salt solutions. For instance, a solution of calcium nitrate inhibits subsequent rubidium absorption to a large extent. Such results can be explained by assuming a decrease of the production or an increase of the decay of a carrier. Did you find similar effects in your experiments? I have seen in Fig.5 that Ca^{++} inhibits Mn^{++} uptake if it is supplied simultaneously. Would it also have such an effect if it had been given previously, say, together with K^{+}?

More in general, could you give a few more details of the conditions under which the ability to absorb Mn^{++} decreases.

ANSWER: I have no data on the possible inhibitory effects of pretreatment with Ca^{++} on the uptake of Mn^{++}. However, from the competitive nature of the inihibition, I would suspect that the Ca^{++} must be present together with the Mn^{++}. With respect to the disappearance of the ability to absorb Mn^{++} only the following data on half times of disappearance are available: at 4°C, 18 hours; at 25°C, 6 to 7 hours; and at 25°C with glucose present, only 2 hours.

СВЯЗЬ ПОТРЕБЛЕНИЯ ДВУХВАЛЕНТНЫХ КАТИОНОВ С ПОТРЕБЛЕНИЕМ КАЛИЯ И ФОСФАТА У ДРОЖЖЕЙ

Ротстейн и Хейс

Исследования, произведенные с Ca^{45}, Sr^{90}, Mn^{54} и P^{32} показывают, что хотя двухвалентные катионы обратимо связываются поверхностью клетки (Ротстейн и Хейс, Arch. Biochem. 63, 87, 1956), они не абсорбируются цитоплазмой, если одновременно не происходит потребления фосфата, связанного с обменом сахаров. В противоположность связыванию катионов поверхностью, которое является относительно неспецифичным, потребление Mg^{2+} и Mn^{2+} одновременно с фосфатом является высоко специфичным. Другие двухвалентные катионы, хотя и слабо адсорбирующиеся, могут конкурентно угнетать потребление Mg^{2+} и Mn^{2+}: Опыты, проведенные с Mn^{54} позволили обнаружить, что однажды адсорбировавшиеся ионы Mn^{2+} уже не обмениваются. Скорость поглощения соответствует кривой сатурационного типа, причем максимальная скорость обуславливается количеством адсорбированного фосфата.

Динитрофенол, азид или арсенат угнетают оба типа поглощения. Заметное стимулирующее действие оказывал калий. При одновременном добавлении K^+, Mn^{2+}, фосфата и глюкозы они поглощаются в следующей последовательности: K^+, PO_4^{3-}, Mn^{2+}. Система, функционирующая в качестве передатчика Mn^{2+}, синтезируется в процессе потребления фосфата, и синтез этот стимулируется калием.