THE ACTIVE TRANSPORT OF Mg^{++} AND Mn^{++} INTO THE YEAST CELL*

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(Received for publication, July 17, 1957)

ABSTRACT

Certain bivalent cations, particularly Mg^{++} and Mn^{++}, can be absorbed by yeast cells, provided that glucose is available, and that phosphate is also absorbed. The cation absorption is stimulated by potassium in low concentrations, but inhibited by higher concentrations. From the time course studies, it is apparent that the *absorption* rather than the presence of phosphate and the potassium is the important factor. Competition studies with pairs of cations indicate that binding on the surface of the cell is not a prerequisite to absorption. The absorption mechanism if highly selective for Mg^{++} and Mn^{++}, as compared to Ca^{++}, Sr^{++}, and UO_2^{++}, whereas the binding affinity is greatest for UO_2^{++}, with little discrimination between Mg^{++}, Ca^{++}, Mn^{++}, and Sr^{++}. In contrast to the surface-bound cations which are completely exchangeable, the absorbed cations are not exchangeable. It is concluded that Mg^{++} and Mn^{++} are actively transported into the cell by a mechanism involving a phosphate and a protein constituent.

The physiological importance of certain bivalent cations, particularly Mg^{++}, Ca^{++}, and Mn^{++}, has been established for many years. In addition, the role of the cations as cofactors in enzyme reactions has been extensively investigated. However, little is known concerning the mechanism by which they are absorbed by cells. In roots of plants bivalent cations are bound in an exchangeable form, and in addition, are absorbed into the "inner space" of cells, in which case, they are no longer exchangeable with extracellular cations (1). In the liver of the rat the uptake of Ca^{++} is related to the age of the rat, part of the Ca^{++} being bound and part ultrafilterable (2). In muscle, the uptake of Ca^{++} is a complex process, apparently involving binding on the surface of the cells, exchange with the intracellular Ca^{++}, as well as an active extrusion proc-

* This paper is based in part on work supported by a grant from the Electro Metallurgical Company, a division of Union Carbide and Carbon Corporation, and in part on work performed under contract with the United States Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, New York.

ess (3, 4). In cells of *Elodea* leaves, Ca^{++} is absorbed by the surface layers of the cell and released under certain conditions into the vacuole (5); the binding substance in this case, and also in the case of *Arbacia* eggs, being ribonucleic acid (6). The cell surface is also involved in the binding of cations by spores (7), bacteria (8), and yeast (9). The binding groups, in the case of yeast, are of two types, phosphoryl and carboxyl (9). Yeast cells can also absorb Mg^{++}, together with K^+ and phosphate, during the metabolism of sugar (10). Some Mg^{++} can also be transported by the K^+ carrier system in the absence of K^+ (11). As in the cells of roots (1), the bivalent cations of yeast cytoplasm are not exchangeable with the cations of the medium, whereas the surface-bound cations are completely exchangeable (9). Exchangeability, therefore, provides a means of distinguishing bound cations from absorbed cations.

The present paper is concerned primarily with the mechanism by which the yeast cell absorbs bivalent cations, particularly the relationship of the absorption mechanism to cation binding by the cell surface and to phosphate and potassium metabolism. The ions studied include Mg^{++}, Ca^{++}, Sr^{++}, UO_2^{++}, and especially Mn^{++}. All the cations except Mg^{++} were studied by isotope technique with Ca^{45}, Sr^{89}, U^{233}, and Mn^{54}, as well as by chemical techniques.

Method

Fresh baker's yeast was thoroughly washed and suspended in a metabolically inert buffer containing triethylamine, tartaric acid, and succinic acids (0.02 M) (12). Unless otherwise noted, the pH was 5.0, and the temperature was 25°C. Aeration and mixing of the suspension were obtained by bubbling washed air through the suspension. In a few anaerobic experiments, N_2 replaced the air. At zero time the appropriate concentrations of the cations (as chlorides) and of phosphate (as triethylamine neutralized phosphoric acid), and glucose were added. In a few cases, when indicated, the glucose and potassium were added 30 minutes before the phosphate and bivalent cations. Samples of the suspension taken at indicated times were centrifuged, and the supernatant medium decanted for analysis. In a number of experiments with Ca^{++} and Mn^{++}, the interactions with the cell were measured by both chemical and isotope techniques. In each case, the results were essentially identical. The same is also true for phosphate (13). For this reason, most of the experiments reported here, except those with Mg^{++} and K^+, were carried out by means of isotope techniques.

Analytical procedures were as follows:—

Phosphate.—Method of Fiske and SubbarRow (14) modified by addition of 15 per cent alcohol prior to color development.

Potassium.—Flame photometric procedure using a Wechselbaum-Varney instrument built by Fearless Camera.

Magnesium.—Precipitation in 20 per cent ethyl alcohol as the magnesium ammonium phosphate in the presence of an excess of phosphate and NH_4OH. The washed precipitate is dissolved in dilute HCl and the phosphate determined. The ratio of P to Mg is 1:1.

Calcium.—Versene titration method of Sobel and Hanok (15).

Manganese.—Permanganate method (16).

Isotopes.—Mn^{54} was counted with a scintillation counter, and P^{32}, Sr^{89}, and Ca^{45} with a Geiger counter. Some double isotope experiments were carried out with Mn^{54} (an γ emitter) and P^{32} (a β emitter). Two procedures were useful: (*a*) counting with a Geiger tube with and without foil inserted above the sample and (*b*) counting first with the Geiger tube and then with the scintillation counter. In each case with appropriate control counts on P^{32} and Mn^{54} standards, the activity for each isotope in the mixture could be readily determined. The statistical counting error was less than 3 per cent in all cases.

RESULTS

Upon addition of cations to a yeast cell suspension, there is a rapid equilibration with binding sites on the surface of the cell, a process characterized in

TABLE I

The Binding and Absorption of Mn^{++} as Influenced by Various Additions

	Mn^{++} uptake, *per cent*		
	2 min.	30 min.	60 min.
Control	25	24	28
+ K^+	23	23	25
+ PO_4	24	23	27
+ K^+ + PO_4	26	26	30
+ Glucose	23	26	28
+ Glucose + K^+	25	28	26
+ Glucose + PO_4	30	44	55
+ Glucose + PO_4 + K^+	33	70	95

The yeast concentration was 100 mg./ml.; Mn^{++}, 7.5×10^{-4} M; PO_4, 5×10^{-3} M; K^+, 1×10^{-2} M; and glucose, 0.3 M.

detail elsewhere (9). For example, in Table I, the surface binding, representing about 25 per cent of the added Mn^{++}, was complete in less than 3 minutes, regardless of other additions to the medium. However, in the presence of phosphate and glucose, there was, in addition to the surface binding, a continuous absorption of Mn^{++}, which was markedly stimulated by K^+. The absorption of Mn^{++} in the presence of phosphate and glucose could be readily differentiated from the surface binding, not only on the basis of its slower rate and its requirement for phosphate and glucose, but also on the basis of its exchangeability. The surface-bound Mn^{++} was completely exchangeable, whereas the absorbed Mn^{++} was not.

Some of the relationships between the absorption of Mn^{++}, K^+, and phosphate are demonstrated in Fig. 1. If all three ions were added simultaneously, together with glucose, the K^+ was rapidly absorbed, being reduced to a low level in 5 minutes. The phosphate was absorbed only after a delay of about 15 minutes (up to 30 minutes in other experiments), at a rate considerably slower than that for K^+. A rapid initial disappearance of Mn^{++} (representing binding

by the cell surface) was followed after a delay period of some 20 minutes by a slow continued disappearance, at a rate about half of that for phosphate. In the sequence of events, phosphate absorption occurred after that of K^+ was completed, and about half of the Mn^{++} was absorbed after the absorption of phosphate was completed. Thus, Mn^{++} uptake is dependent, not on the presence of K^+ and phosphate, but on their prior absorption. For this reason, it is possible to separately expose the cells to each of the ions in question. For example, the broken lines of Fig. 1 represent an experiment in which cells were

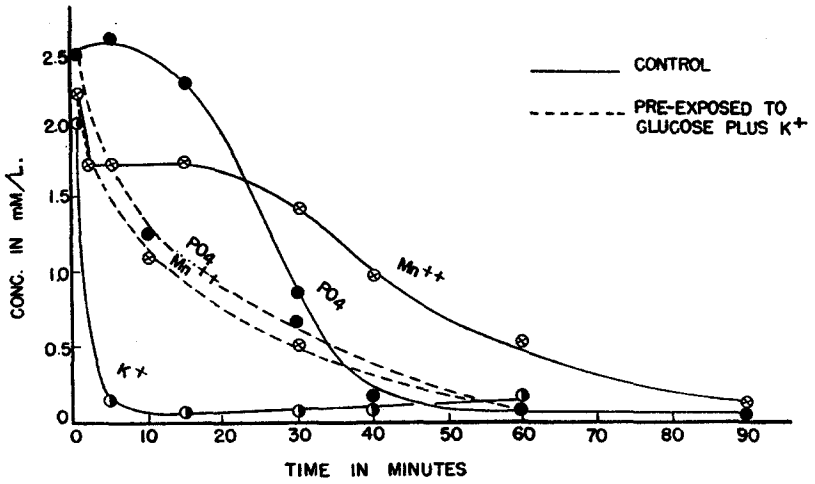

FIG. 1. The disappearance of K^+, Mn^{++}, and phosphate from the medium of respiring yeast cells. The yeast concentration was 100 mg./ml. and glucose, 0.1 M. In the *control* the glucose, K^+, Mn^{++}, and phosphate were added at zero time. In the *preexposed* the K^+ and glucose were added 30 minutes before zero time.

preexposed to K^+ and glucose. Such cells absorbed phosphate and Mn^{++} in approximately a 1:1 ratio, with no delay period. In another paper (17), experiments are described in which cells, preexposed to K^+, phosphate, and glucose, when washed, are later able to absorb Mn^{++}, if given glucose.

Information on the interrelationships among K^+, phosphate, and Mn^{++} uptakes was obtained by a series of experiments in which two of the ions were maintained at a constant concentration, and the third was varied. In each case, the absorption of Mn^{++} was measured, with appropriate corrections for the surface binding obtained from control experiments with no glucose.

The influence of phosphate concentration is demonstrated in Fig. 2. With no phosphate present, Mn^{++} absorption was essentially zero, but with phosphate present, increasing concentrations were associated with an increased rate of Mn^{++} absorption. In the experiment of Fig. 2, and in others not shown,

the initial rate of Mn^{++} uptake was approximately proportional to the phosphate concentration except with concentrations above 10 mM/liter, in which case, less Mn^{++} was absorbed than would be expected on the basis of proportionality. The highest rate of Mn^{++} uptake in the experiment of Fig. 2 was 0.03 M per kilo of cells per hour.

The rate of Mn^{++} uptake is also conditioned by the concentration of Mn^{++} (Fig. 3), but not to nearly the same extent as in the case for phosphate. With the lowest concentrations (0.5 and 1.0 mM), the uptake was completed during

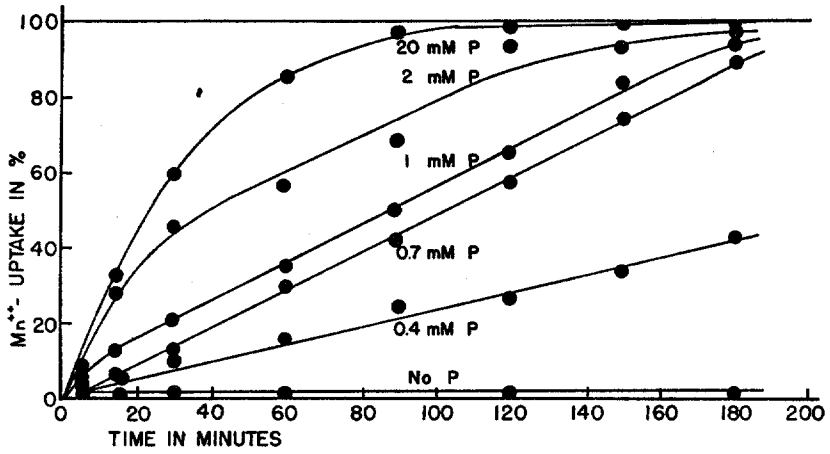

FIG. 2. The influence of phosphate concentration on the absorption of Mn^{++} by respiring yeast. The yeast concentration was 50 mg./ml.; Mn^{++}, 1×10^{-3} M; K^+, 5×10^{-4} M; and glucose, 0.1 M. Cells were preexposed to K^+ and glucose for 30 minutes. Corrections were made for surface binding, by controls with no glucose.

the course of the experiment. With higher concentrations, an initial rapid uptake of about 30 minutes' duration was followed by a prolonged period of uptake at a constant but slower rate.

Although Mn^{++} uptake is completely dependent on phosphate uptake, the reverse situation does not hold. Phosphate uptake proceeds to the same extent in the presence or absence of Mn^{++} with only a slight reduction in the rate if the Mn^{++} concentration is relatively high. Also, as pointed out previously, the phosphate uptake may precede the Mn^{++} uptake by several hours. For these reasons the relationship between the uptakes of the two ions is complex. Phosphate, if present in higher concentration than the Mn^{++}, is absorbed more rapidly. On the other hand, Mn^{++}, when present in equal or higher concentrations, is never absorbed more rapidly than is the phosphate, although it may be absorbed at about the same rate under some circumstances (see Fig. 1). However, the absorption of the Mn^{++} may continue after all the phosphate is

absorbed, and after a period of time, may exceed the phosphate absorption. For example, in the experiment of Fig. 2, the Mn^{++} concentration was 1×10^{-3} M. By 180 minutes nearly all was absorbed with a phosphate concentration of only 0.7×10^{-3} M. By 8 hours (not shown on graph), all was absorbed with a phosphate concentration of only 0.4×10^{-3} M. Similarly in the experiment of Fig. 3, yeast exposed to the highest concentration of Mn^{++} absorbed more Mn^{++} than phosphate. The ratio of total Mn^{++} absorbed to total phosphate absorbed rarely exceeded 2 to 1 in any experiment, with no fixed stoi-

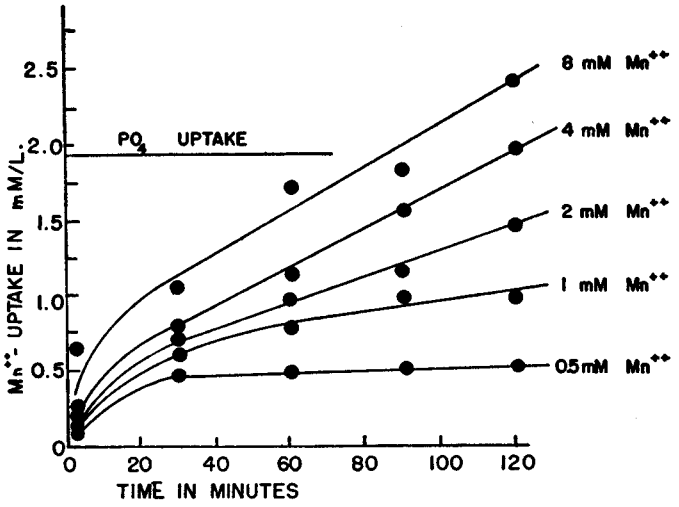

FIG. 3. The influence of Mn^{++} concentration on the absorption of Mn^{++} by respiring yeast. The yeast concentration was 50 mg./ml.; phosphate, 2×10^{-3} M; K^+, 2×10^{-3} M; and glucose, 0.1 M. The cells were preexposed to K^+ and glucose for 30 minutes. Corrections were made for surface binding, by controls with no glucose.

chiometric relationship apparent either in terms of rates of uptake, or total amounts absorbed.

The pattern in the case of K^+ was quite different (Fig. 4). Absorption of Mn^{++} occurred in the absence of K^+, but was markedly stimulated in its presence, provided that the concentration was not too high. Maximal stimulation was obtained at 3×10^{-3} M, with concentrations higher than 2×10^{-2} M being markedly inhibitory. The stimulatory action of K^+ on the absorption of Mn^{++} is directly referable to its marked stimulatory action on the absorption of phosphate (13). On the other hand, the inhibitory effects of higher concentrations of K^+ are not referable to any direct effect on phosphate uptake, for the latter process is stimulated by all K^+ concentrations up to 0.1 M. Nor is any direct interaction of extracellular K^+ and Mn^{++} on the cell apparent. For example, the inhibitory effect persists in cells previously exposed to high con-

centrations of K^+ plus glucose, then washed free of K^+. Furthermore, previous studies have demonstrated that Mn^{++} has no effect on the ability of cells to absorb K^+ (18). It is therefore concluded that the inhibitory effects are related to the elevated cellular content of K^+ resulting from exposure to K^+ and glucose (20).

The mechanism for the absorption of Mn^{++} is highly specific. Of the bivalent cations tested, Mg^{++} and Mn^{++} are the only two that were absorbed at an appreciable rate, the former being absorbed about three times as rapidly at

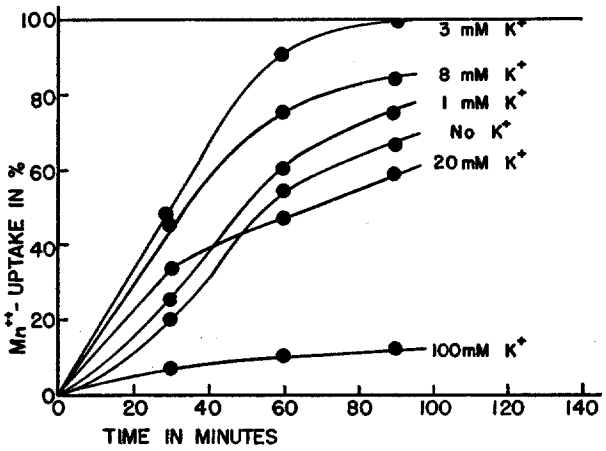

FIG. 4. The influence of K^+ concentration on the absorption of Mn^{++} by respiring yeast. The yeast concentration was 100 mg./ml.; phosphate, 7.5×10^{-4} M; Mn^{++}, 7.5×10^{-4} M; and glucose, 0.1 M. Corrections were made for surface binding, by controls with no glucose.

equal concentrations (Fig. 5). Of the others, Ca^{++} was absorbed at about one-tenth the rate of Mg^{++}, while Sr^{++} and UO_2^{++} (not shown on Fig. 4) were absorbed at only a very slow rate, if at all.

In order to investigate the specificity of the reaction in more detail, a series of competition experiments were carried out in which Mn^{++} uptake was measured in the presence of several concentrations of UO_2^{++}, Ca^{++}, or Mg^{++}. Representative data are shown in Fig. 6. The concentrations of Mn^{++} and of yeast cells were chosen so that approximately 20 per cent of the added Mn^{++} was bound by the cell surface, represented by the data for "no glucose." In the control (no competing ion), the Mn^{++} binding was also 20 per cent as indicated by the point at 2 minutes. Thereafter, Mn^{++} was absorbed to completion at 90 minutes. In the presence of UO_2^{++}, at ten times the Mn^{++} concentration (3×10^{-3} M compared to 3×10^{-4} M), the initial binding of Mn^{++} was almost completely blocked. Nevertheless, absorption proceeded at essentially the same rate as in the absence of UO_2^{++}. In the presence of Ca^{++}, at three times

the Mn^{++} concentration, the binding of the latter ion was blocked about 60 per cent and the rate of absorption was inhibited about 40 per cent. In the presence of equimolar Mg^{++} and Mn^{++}, the binding of the latter ion was only

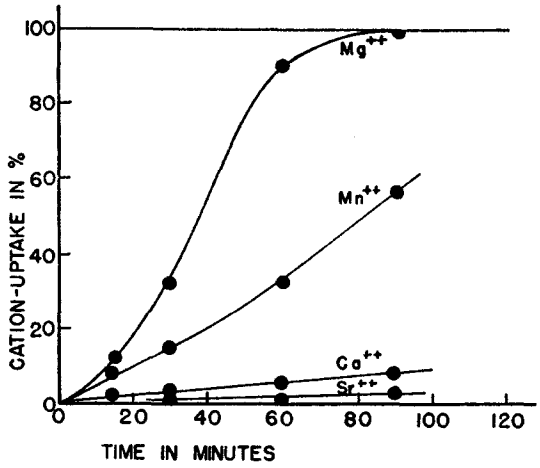

FIG. 5. The specificity pattern in the absorption of bivalent cations by respiring yeast. The yeast concentration was 50 mg./ml.; all ions, 5×10^{-4} M; glucose, 0.1 M. Corrections were made for surface binding, by controls with no glucose.

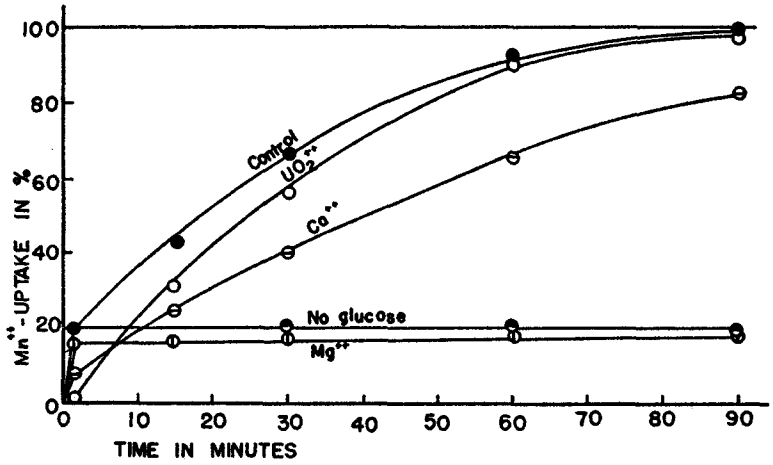

FIG. 6. Competitive inhibition of Mn^{++} binding and absorption by other cations. The yeast concentration was 100 mg./ml.; Mn^{++}, 3×10^{-4} M; UO_2^{++}, 3×10^{-3} M; Ca^{++}, 1×10^{-3} M; Mg^{++}, 3×10^{-4} M; K^{+}, 1×10^{-3} M; and glucose, 0.1 M.

slightly blocked but the absorption was almost completely inhibited. These data indicate that the surface binding is not a necessary prerequisite to absorption. Furthermore, the specificity pattern of the two processes is entirely

different. UO_2^{++} has a high affinity for the surface-binding sites and essentially none for the absorption sites; Ca^{++} has only a slightly greater affinity for the surface-binding sites than for the absorption sites; and Mg^{++} has a greater affinity for the absorption sites. The data of Fig. 6 are in essential agreement with the specificity pattern for absorption shown in Fig. 5, and with that for surface binding studied previously (9).

The absorption of Mn^{++} was found to be the same under anaerobic as under aerobic conditions, just as in the case of phosphate absorption (13). The absorption of Mn^{++} also varies with pH, but again the effects are referable to parallel effects of pH on phosphate absorption.

DISCUSSION

In the yeast cell, the mechanism for absorption of bivalent cations possesses many of the properties of an active transport system: (*a*) a high degree of specificity, favoring Mg^{++} and Mn^{++}; (*b*) a dependence on metabolism of external substrate; (*c*) competition between cations for the transporting mechanism; and (*d*) directional asymmetry, with a high rate of influx, and essentially zero efflux. It is not known whether the cations can be moved against the activity gradient, because the concentration of free Mn^{++} or Mg^{++} within the cytoplasm is not known, most of the bivalent cations of the cell existing as complexes with proteins and phosphate compounds (19).

The actions of K^+ and of phosphate on bivalent cation absorption are of considerable interest. In each case, it must be kept in mind that the absorption of the ion, rather than its presence, is the prerequisite factor. The phosphate dependence is related to the chemical nature of the "carrier" for the transport of the bivalent cations. In another study (17), it will be shown that this carrier is a phosphorylation product, which is synthesized during the absorption of phosphate, and which decays at a rate determined by the metabolic state of the cell.

In the case of K^+, two effects are seen, a stimulating action at lower concentrations and an inhibiting action at higher concentrations. Both effects are probably related to the over-all electrolyte balance of the cell. In the absence of phosphate, yeast cells absorb K^+ in a stoichiometric exchange for H^+ derived primarily from metabolic reactions (20, 21). Part of the K^+ is balanced against metabolic anions, such as succinate and bicarbonate, but part is at the expense of cellular buffer capacity, resulting in alkalinization of the cytoplasm. In the absence of K^+, phosphate is absorbed, but only to a limited extent (13). Because the $H_2PO_4^-$ ion is selected in preference to HPO_4^{--}, the absorption is again at the expense of cellular buffer, resulting, in this case, in acidification. In the presence of both K^+ and $H_2PO_4^-$, the absorption of the latter is markedly enhanced, even though the absorption of K^+ occurs first. Thus, a cell rich in fixed base (K^+) can absorb much greater quantities of $H_2PO_4^-$. In turn, with greater absorption of $H_2PO_4^-$, the synthesis of the Mn^{++} carrier is

enhanced, accounting for the stimulation of Mn^{++} absorption. However, with a large excess of K^+ compared to phosphate (as in the experiment of Fig. 4), even though the phosphate uptake and carrier synthesis are enhanced, the cell has such a large surplus of cation that the absorption of Mn^{++} is inhibited.

The nature of the transporting reaction is not known. However, some of the observations in the present paper are suggestive: (*a*) the surface binding by fixed negative groups of the cell surface is not involved; (*b*) the reaction is effectively irreversible; (*c*) the carrier is a phosphorylation product; (*d*) the reaction is dependent on metabolism; and (*e*) the specificity pattern is remarkably like that of phosphorylation enzymes with Mg^{++} requirements, rather than a binding to fixed ionic groups, such as phosphoryl or carboxyl. Perhaps, then, the carrier system involves, in addition to a phosphorylated substance, a protein constituent, the two together conferring the specificity. A hypothesis which might account for the properties listed above is that of the contractile protein of the cell membrane, proposed by Goldacre and elaborated by Danielli (22).

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