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ENERGY-DEPENDENT Mn^{2+} AND Ca^{2+} UPTAKE BY THE EMBRYONIC CHICK CHORIOALLANTOIC MEMBRANE

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SUMMARY

The chick chorioallantoic membrane is an epithelial tissue which actively transports large amounts of Ca^{2+} during embryonic development. In this paper Mn^{2+} uptake by the tissue was studied and compared to Ca^{2+} uptake in parallel experiments. The purpose of these experiments was to determine if Mn^{2+} could be used as a paramagnetic analog for Ca^{2+} so that electron paramagnetic resonance could be used to gain more information about the Ca^{2+} transport system.

It was found that Mn^{2+} uptake was reduced significantly under conditions that reduced Ca^{2+} uptake and that Mn^{2+}, like Ca^{2+}, was taken up preferentially by the ectodermal side of the tissue.

Mn^{2+} uptake showed saturation kinetics with a K_m of 0.33 mM. Mn^{2+} uptake was also competitively inhibited by Ca^{2+}, and Ca^{2+} uptake inhibited by Mn^{2+}.

Electron microprobe studies showed that Mn^{2+} was localized in the ectoderm of the tissue in the same way as Ca^{2+}. It was concluded from these studies that significant amounts of Mn^{2+} were accumulated by the active Ca^{2+} transport mechanism and that Mn^{2+} could be a useful paramagnetic probe of divalent cation transport in this tissue.

INTRODUCTION

Many epithelial tissues such as intestine, kidney, mammary gland and avian shell gland are involved in the transcellular movement of large amounts of Ca^{2+} [1]. Since high concentrations of intracellular Ca^{2+} may be detrimental to cell function [2, 3], the bulk movement of Ca^{2+} must presumably be accomplished without raising the intracellular Ca^{2+} level of the transporting cells above about 10^{-6} M [4]. The pathway of Ca^{2+} through these tissues and the molecular mechanism of translocation are not known. The transport mechanism may be a modification of normal cell Ca^{2+} homeostatic mechanisms, such as the plasma membrane pump and mitochondrial uptake [5], or it may involve a separate process.

The chorioallantoic membrane of the chick is another epithelial tissue which has an active Ca^{2+} transport mechanism. It is a convenient model system for studying transepithelial transport and is relatively simple morphologically. The chorioallantoic membrane lines the shell of the developing embryo and actively transports large amounts of Ca^{2+} into the embryonic circulation [6]. In vitro Ussing chamber experiments have shown that the membrane transports large amounts of Ca^{2+} from ectoderm to endoderm with little backflux [6], that the transport mechanism is energy dependent [6], and that the transport process stimulates oxygen consumption [7]. Studies with the electron-probe X-ray microanalyzer have shown high local concentrations of Ca^{2+} in the ectodermal cells of the tissue under transport conditions [8, 9]. These and other results have suggested that Ca^{2+} may be sequestered from the rest of the cell contents as it is transported. It has been postulated that special endocytotic vesicles are formed for the transport of Ca^{2+} across the tissue [9].

The purpose of the investigations reported in this paper is to determine the extent to which Mn^{2+} is an analog for Ca^{2+} in the chorioallantoic membrane so that electron paramagnetic resonance (EPR) spectroscopy may be applied to the study of Ca^{2+} transport in this tissue. EPR can give specific information on the physical environment of divalent cations in biological systems. To study Ca^{2+}-transporting systems, the paramagnetic ion Mn^{2+} is substituted for Ca^{2+}, which is not paramagnetic. This approach has been successfully used to study Ca^{2+} uptake by isolated mitochondria [10], which transport Mn^{2+} as an analog to Ca^{2+}.

To compare directly Mn^{2+} with Ca^{2+} uptake, several different experimental approaches have been used. The effect of inhibitors has been investigated in tissue uptake experiments, and transport chamber studies have been used to look at unidirectional uptake and transmembrane fluxes. Kinetic studies have investigated competitive inhibition between Mn^{2+} and Ca^{2+}, and the distribution of Mn^{2+} in the tissue was studied in the electron microprobe. From these studies it is concluded that a significant amount of Mn^{2+} enters the tissue by the same energy-dependent system that transports Ca^{2+}. The following paper [11] presents the results of the EPR studies of Mn^{2+} in the transport system.

MATERIALS AND METHODS

Preparation of membranes

The chorioallantoic membranes used in these experiments were taken from fertile White Leghorn eggs purchased from Spafas, Inc. (Norwich, Conn.). All membranes were from the air space region of the egg and had the inner shell membrane attached to the chorioallantoic membrane itself. 16 to 18-day eggs were used unless otherwise noted, since Ca^{2+} transport is at a maximum during this period [6].

Solutions

All experiments used a standard buffer solution of 140 mM Na^+, 5 mM K^+, 0.5 mM Mg^{2+}, 25 mM $Tris^+$, 170 mM Cl^- and 0.5 mM SO_4^{2-} with a pH of 7.4 at 37 °C. $MnCl_2$ and $CaCl_2$ were added in the concentrations indicated. All salts were of reagent grade. Phosphate was not included in the solutions since it precipitated when Mn^{2+} was added. Leaving out phosphate had no observable effect on Mn^{2+} or Ca^{2+} uptake.

Experimental procedures

Two basic experimental procedures were used to study Mn^{2+} and Ca^{2+} accumulation by the chorioallantoic membrane. One procedure was to mount the membrane in an Ussing-type transport chamber with the same concentration of ion on each side. By adding radioactive Mn^{2+} or Ca^{2+} to one side, one could monitor unidirectional radioactive tracer movement into and across the tissue. The experimental procedure used was similar to one that has been previously described [6]. In this type of experiment, the amount of labeled divalent cation in the membrane at the end of the experiment is referred to as "tissue uptake" and the amount of ion that moves across the membrane is called the "transmembrane flux". In these experiments the concentration of Mn^{2+} or Ca^{2+} was 0.25 mM on each side, and the experiments were run for 3 h.

The second procedure used was the uptake experiment [12]. Several pieces of chorioallantoic membrane 2 cm^2 in area were cut from the same egg and washed briefly in buffer. Each piece was placed in a separate 30 ml. beaker containing 5 ml of buffer with 0.75 mM of radioactively labeled Mn^{2+} or Ca^{2+} added. The beakers were shaken for 1.5 h in a Dubnoff Metabolic Shaking Incubator (Precision Scientific Co., Chicago, Ill.) at 37 °C. The membranes were then washed twice in buffer for 1 min and counted. When the inhibitors 2,4-dinitrophenol, oligomycin and rotenone were used, the membranes were preincubated for 15 min in buffer solution with only the inhibitor present. They were then exposed to the labeled Mn^{2+} or Ca^{2+} buffer solution with inhibitor still present. Control experiments showed that the 15 min pre-incubation and the alcohol solvent used for the metabolic inhibitors had no effect on membrane uptake.

The kinetic studies of Mn^{2+} and Ca^{2+} entry into the chorioallantoic membrane were modified uptake experiments. Four pieces of membrane were cut from each egg and pre-incubated for 5 min in buffer. The pieces were incubated with labeled Mn^{2+} or Ca^{2+} in the Dubnoff Metabolic Shaker for 10 s, 1, 2 and 4 min, post-washed twice for 1 min in buffer, and counted. The slope of the uptake curve over the first 2 min was taken as the initial velocity of entry. The average of at least six eggs was used to determine the initial velocity at each concentration of Mn^{2+} or Ca^{2+}.

Counting procedures

The ^{54}Mn and ^{45}Ca isotopes were obtained from Amersham/Searle (Des Plaines, Ill.). Membranes to be analyzed for ^{45}Ca were solubilized in 1 ml of NCS tissue solubilizer (Amersham/Searle) for 3 h at 37 °C in the Dubnoff shaker. 10 ml of scintillation fluid were added, and the membranes counted by standard scintillation techniques. To measure ^{45}Ca isotope fluxes in the Ussing chamber experiments, 0.1 ml samples were taken from each side, dissolved in 5 ml of acid alcohol, and counted after adding scintillation fluid. Membranes containing ^{54}Mn were counted directly in a well-type gamma spectrometer without solubilization. The 0.1 ml Ussing chamber samples containing ^{54}Mn were brought to 1 ml with distilled water and counted directly.

Electron microprobe techniques

Tissues which were to be examined for Mn^{2+} in the electron probe X-ray

microanalyzer were exposed to 0.75 mM Mn^{2+} for 1.5 h using the Dubnoff shaker uptake procedure. The membranes were then fixed for 30 min with 4 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) containing 1 mM sodium pyrophosphate. This was followed by a short wash in buffer, a 30 min post-fixation in 1 % osmium tetroxide in the same buffer, dehydration in ethanol, and embedding in plastic. 1 μm thick sections of the tissue were cut with glass knives without exposure to water and mounted on silicon wafers. These were examined with an EMX Electron Probe X-ray Microanalyzer (Applied Research Laboratories, Inc., Glendale, Calif.) using a 22 kV accelerating potential and about a $7 \cdot 10^{-9}$ A sample current. The beam diameter was less than 1 μm. Images were recorded from the oscilloscope screen on Polaroid® film. This procedure is almost identical to the one earlier used to study Ca^{2+} in the chorioallantoic membrane [8, 9], except that 1 mM pyrophosphate has been substituted for the 1 % oxalate. Experiments with radioactive Mn^{2+} have shown that no substantial loss of Mn^{2+} occurs from tissue fixed in the presence of pyrophosphate.

RESULTS

Transepithelial fluxes of Mn^{2+} and Ca^{2+}

Table I shows the results of transport chamber studies comparing the unidirectional fluxes of Mn^{2+} with that observed for Ca^{2+}. Experiments A and B were carried out with equimolar concentrations (0.25 mM) of Ca^{2+} or Mn^{2+} on both sides of the chamber. Tissue uptake of both ions was considerably greater in the forward direction, i.e. by the ectodermal side of the membrane, as compared to uptake into the tissue from the endodermal bathing solution (backflux). Ectodermal uptake exceeded endodermal uptake by at least 5-fold. This preferential tissue uptake by the ectoderm is also shown in Experiment C of Table I. These are the results of a study in which 0.25 mM Mn^{2+} (plus ^{54}Mn) was placed on one side of the membrane with an

TABLE I

UNIDIRECTIONAL FLUXES OF Ca^{2+} AND Mn^{2+} IN TRANSPORT CHAMBER EXPERIMENTS

"Tissue uptake" is the amount of ion that accumulated within the tissue during the 3 h experiment and "trans-membrane flux" is the amount of ion that completely crossed the mounted tissue during the same period. The "forward" direction is ion flux from the ectodermal bathing medium into and across the tissue; "backflux" is movement from the endodermal bathing medium. Results are in nmol/cm² per h and expressed as the mean of six experiments ±S.E. "Flux ratio" is the mean of the forward flux divided by the mean of the back flux. In experiments A and B equimolar concentrations of Ca^{2+} or Mn^{2+} (0.25 mM) were present on both sides of the membrane. In experiment C 0.25 mM Mn^{2+} (with tracer ^{54}Mn) was placed on one side of the tissue with 0.25 mM Ca^{2+} on the opposite side so that the down-hill chemical gradient for Mn^{2+} from ectoderm to endoderm (forward) or endoderm to ectoderm (backflux) was identical in each case.

Experiment	Tissue uptake			Transmembrane flux		
	Forward	Backflux	Flux ratio	Forward	Backflux	Flux ratio
A. Ca^{2+} both sides	5.4±1.6	0.4±0.1	13.5	7.4±1.7	0.9±0.5	8.2
B. Mn^{2+} both sides	5.5±0.7	0.8±0.1	6.5	9.9±1.4	7.4±2.4	1.3
C. Mn^{2+} one side	6.9±0.9	0.8±0.1	8.6	6.6±1.7	1.7±0.9	3.4

equimolar concentration of unlabeled Ca^{2+} on the opposite side. Thus an identical downhill gradient for Mn^{2+} was established either from ectoderm to endoderm, or from endoderm to ectoderm. It can be seen that even under these conditions backflux of Mn^{2+} into the tissue was small, while Mn^{2+} uptake by the ectoderm was unchanged and 8.6 times greater than endodermal Mn^{2+} uptake.

In these experiments the amount of Mn^{2+} or Ca^{2+} that completely traversed the tissue during the 3 h period of study was also monitored. These data are shown as transmembrane flux in Table I. With Ca^{2+} on both sides of the membrane (Experiment A), over 8 times as much Ca^{2+} was transferred from the ectodermal to the endodermal bathing solution as compared to movement in the opposite direction. With Mn^{2+} in the donor solution and Ca^{2+} in the acceptor solution (Experiment C) flux in the forward direction was also much larger than backflux. Under conditions where there were equimolar amounts of Mn^{2+} on both sides of the membrane (Experiment B) Mn^{2+} backflux was relatively large compared to Ca^{2+} under similar conditions or when the large down-hill chemical gradient was established. In other experiments, this large transmembrane backflux was not affected by metabolic inhibitors, whereas significant reductions in the forward flux of Mn^{2+} could be demonstrated, suggesting an increase in passive permeability of the whole tissue under these particular conditions.

Uptake of Mn^{2+} and Ca^{2+}

Tissue uptake of Mn^{2+} and Ca^{2+} by the membrane was studied in parallel experiments in the Dubnoff Metabolic Shaker. Table II shows that Ca^{2+} uptake into the tissue was decreased to at least one-third of the control value by the metabolic inhibitors 2,4-dinitrophenol, oligomycin and rotenone. Membranes that have been "stripped", which is a mechanical separation of the inner shell membrane from the chorioallantoic membrane before incubation, and membranes taken from 10-day-old

TABLE II

EFFECT OF INHIBITORS ON TISSUE UPTAKE OF Ca^{2+} AND Mn^{2+} IN METABOLIC SHAKER EXPERIMENTS

Membranes were incubated in 0.75 mM Ca^{2+} or Mn^{2+} for 1.5 h. Results are expressed in nmol/cm² ±S. E. for 8 experiments except for controls which represent 32 experiments. The concentration of metabolic inhibitor used is given in the table. Chorioallantoic membranes from 10-day-old embryos are histologically immature; "stripped membranes" are from 15- to 17-day-old embryos with the inner shell membrane mechanically removed from the tissue before study.

Experiment	Tissue Uptake			
	Calcium		Manganese	
	nmol/cm²	% of control	nmol/cm²	% of control
Control	36.4±3.0	—	30.5±2.1	—
2,4-dinitrophenol (0.2 mM)	11.6±0.9	31.8	20.2±3.4	66.2
Oligomycin (10 μg/ml)	7.3±0.9	19.9	15.5±0.2	50.8
Rotenone (10 μg/ml)	8.2±1.9	22.4	18.1±1.3	59.3
10-day membranes	7.4±0.9	20.3	17.1±1.2	56.1
Stripped membranes	6.0±1.8	16.5	21.9±1.9	71.8

eggs, which have not yet begun to actively transport Ca^{2+} from the egg shell, also showed little Ca^{2+} accumulation. These results are the same as those found by studying Ca^{2+} transport in Ussing chambers [6].

Table II also shows Mn^{2+} uptake under the same conditions as the Ca^{2+} uptake experiments. Control membranes took up somewhat less Mn^{2+} than Ca^{2+}. This uptake was also significantly inhibited by 2,4-dinitrophenol, oligomycin and rotenone (t-test, $p < 0.01$), although the effect was not as great as that seen with Ca^{2+}. Similarly, 10-day membranes and stripped membranes contained less Mn^{2+} than the controls.

Kinetics of Mn^{2+} and Ca^{2+} uptake

A study was made of the kinetics of Mn^{2+} and Ca^{2+} uptake into the chorio-allantoic membrane to determine the saturability and affinity of the transport system for these ions. Competition studies between Mn^{2+} and Ca^{2+} were done to determine the effect of one ion on the uptake of the other. The competitive or noncompetitive nature of the Mn^{2+}-Ca^{2+} interaction was evaluated from these experiments.

The kinetics of uptake into the membrane were similar for both Mn^{2+} and Ca^{2+} (Fig. 1). As can be seen in Fig. 1A, which is a typical uptake curve for Mn^{2+} alone and Mn^{2+} in the presence of Ca^{2+}, there was a very rapid binding of Mn^{2+} to the membrane within the first 10 s. The uptake was then linear for at least 2 min. The uptake of Ca^{2+}, and of Ca^{2+} in the presence of Mn^{2+} also showed the same pattern (Fig. 1B). The initial velocity of entry (v_i) of Mn^{2+} or Ca^{2+} into the tissue was taken as the slope of the uptake curve during the first 2 min. Within the physiological concentration range 0.1–1.0 mM, values for v_i were determined for each ion alone and in the presence of the other. v_i for Ca^{2+} saturated at high concentrations and was reduced by the presence of Mn^{2+} at all Ca^{2+} concentrations. The v_i of Mn^{2+} followed a similar pattern as a function of Mn^{2+} concentration. It also saturated and was inhibited by Ca^{2+}.

These data of v_i as a function of concentration may be replotted according to the method of Lineweaver and Burk [13] for Mn^{2+} and Ca^{2+} (Fig. 2). Assuming that

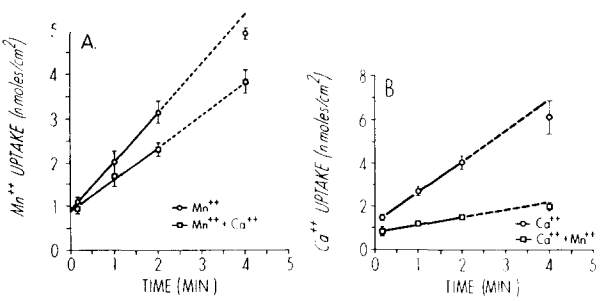

Fig. 1. Typical Mn^{2+} and Ca^{2+} tissue uptake experiments as a function of time. Plot A shows the uptake of 0.5 mM Mn^{2+} alone and in the presence of 5 mM Ca^{2+}. The corresponding experiment with 0.5 mM Ca^{2+} in the presence and absence of 5 mM Mn^{2+} is shown in plot B. Note that ordinate B is different from ordinate A. Each point represents the mean and standard error of at least six experiments. Initial velocity of entry into the tissue, v_i, was taken as the slope of the linear least-squares line through the first three points (solid line).

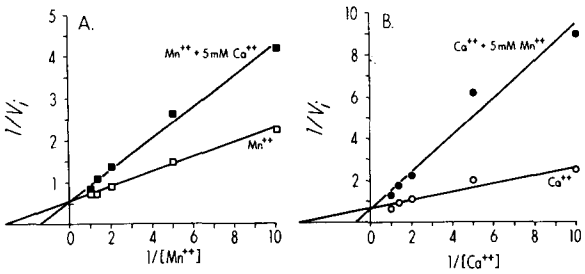

Fig. 2. Lineweaver-Burk plots of v_i as a function of external ion concentration. In this type of plot, the Michaelis constant K_m is given by the negative reciprocal of the abscissa intercept, and the maximum velocity V is the reciprocal of the ordinate intercept. Fig. A shows Mn^{2+} uptake with and without Ca^{2+} present, and Fig. B shows Ca^{2+} uptake with and without Mn^{2+}. Lines through data points were obtained from linear least-squares analysis. Note that the ordinates of A and B are different.

TABLE III

Mn^{2+} AND Ca^{2+} UPTAKE KINETIC PARAMETERS DERIVED FROM LINEWEAVER-BURK PLOTS OF v_i SHOWN IN FIG. 2

K_m is the apparent Michaelis constant expressed as mM. V is the maximum velocity of uptake in nmol/cm² per min. Values for Ca^{2+} uptake studied over a 3 h period are taken from the data of Garrison and Terepka [12].

Experiment	K_m	V
Mn^{2+}	0.33	1.90
Mn^{2+} + 5 mM Ca^{2+}	0.67	1.81
Ca^{2+}	0.29	1.50
Ca^{2+} + 5 mM Mn^{2+}	1.32	1.51
Ca^{2+} (3 h)	0.28	1.83

the uptake of these ions can be described by Michaelis-Menten kinetics, the intercepts of the line determined by these points give K_m, the apparent Michaelis constant, and V, the maximum velocity of uptake. In Fig. 2A, it can be seen that the lines for Mn^{2+}, and Mn^{2+} plus Ca^{2+}, intersect at the ordinate indicating that the V of Mn^{2+} uptake does not change significantly with Ca^{2+} present but the K_m does. The V and K_m of Ca^{2+} uptake with and without Mn^{2+} present (Fig. 2B) follows the same pattern. These data, summarized in Table III, are consistent with competitive inhibition between Mn^{2+} and Ca^{2+} for the uptake sites. The values obtained for Ca^{2+} uptake alone (Table III, third line) agree very well with those obtained previously [12] measuring total uptake by the ectodermal side of the membrane in the Ussing chamber over a 3-h period (Table III, last line). These Mn^{2+} and Ca^{2+} uptake experiments were also analyzed using Eadie-Hostee plots to determine K_m and V, and similar results were obtained.

Electron microprobe analysis of Mn^{2+} distribution

Electron probe X-ray microanalyzer studies of the Ca^{2+} distribution in the chorioallantoic membrane after exposure to Ca^{2+} shows Ca^{2+} localized in high

concentrations in certain regions of the tissue [8, 9]. This is in contrast to the distribution of other elements such as phosphorus and sulfur, which show a diffuse distribution throughout the membrane [14]. These Ca^{2+} localizations were found only in the "capillary-covering" cells of the chorioallantoic membrane ectoderm and were not present in young membranes or in membranes incubated with nitrogen or metabolic inhibitors.

If Mn^{2+} is accumulated by the Ca^{2+} transport system of the chorioallantoic membrane, then it might be expected that the pattern of Mn^{2+} distribution in the membrane would be similar to that of Ca^{2+}. For this reason, tissues exposed to Mn^{2+} were examined in the electron microprobe. Sample current and Mn^{2+} X-ray images of membranes prepared as described in Methods above were recorded. Although more difficult to detect than Ca^{2+}, when Mn^{2+} X-ray signals were detected they eminated primarily from discrete locations within the tissue samples. When the electron beam was centered on these locations, the counts from the X-ray spectrometer set for Mn^{2+} were from 5 to 15 times higher than the Mn^{2+} counts seen elsewhere in the tissue, indicating high local concentrations of Mn^{2+}. By comparing the position of these localizations in the X-ray images with the corresponding sample current images, the Mn^{2+} concentrations were localized to the chorioallantoic membrane ectoderm and appeared to be near the ectodermal capillaries. This distribution is similar to the discrete distribution of the Ca^{2+} characteristic of actively transporting chorioallantoic membranes [9]. Also, like Ca^{2+}, no Mn^{2+} concentrations were seen in the mesodermal or endodermal regions of the tissue.

DISCUSSION

The purpose of these investigations was to determine whether Mn^{2+} was taken up by the chorioallantoic membrane using the same energy-dependent mechanism that transports Ca^{2+}. The tissue uptake and transport chamber experiments, in which the Mn^{2+} results in general paralleled those of Ca^{2+}, indicated that a significant quantity of Mn^{2+} was accumulated by an energy-dependent process. The kinetic studies yielded similar kinetic parameters for Mn^{2+} and Ca^{2+} uptake and showed competitive inhibition of the uptake of one ion by the other. Mn^{2+} and Ca^{2+} were shown in electron probe investigations to have similar distributions in the membrane. On the other hand, these studies revealed several differences between Mn^{2+} and Ca^{2+} that may be important in understanding divalent cation transport in this tissue.

In the transport chamber experiments, where equimolar amounts of the ions were present on both sides of the membrane (Table I), the pattern of tissue uptake of Mn^{2+} was very similar to the pattern of uptake of Ca^{2+}, with much greater uptake by the ectoderm than the endoderm in both cases. However, the transmembrane flux of Mn^{2+} in each direction was almost equal, while there was a larger forward flux than backflux with Ca^{2+}. It may be that when the membrane is exposed to Mn^{2+} on both sides for 3 h it gradually becomes permeable to Mn^{2+} and passive fluxes develop in both directions across the tissue. This is supported by the fact that when Ca^{2+} replaced Mn^{2+} on one side, the values for both tissue uptake and transmembrane flux of Mn^{2+} were close to those seen for Ca^{2+}, when Ca^{2+} was present on both sides of the membrane. In this case the transmembrane flux for Mn^{2+} in the forward

direction was much larger than the backflux, even though a large chemical gradient existed on both cases that favored Mn^{2+} movement across the membrane.

In the tissue uptake studies (Table II) Mn^{2+} uptake was significantly reduced in all experiments. The inhibition by 2,4-dinitrophenol, oligomycin and rotenone suggests that much of the Mn^{2+} uptake is dependent on ATP derived from oxidative phosphorylation. It is interesting in this respect that both Mn^{2+} and Ca^{2+} stimulate oxygen consumption in the chorioallantoic membrane [7]. The inhibition of Mn^{2+} uptake was not as great as the inhibition seen with Ca^{2+}, so that relatively more Mn^{2+} may be entering the tissue passively.

The kinetic studies of Mn^{2+} and Ca^{2+} uptake by the chorioallantoic membrane showed rapid initial binding to the membrane followed by linear uptake in the case of both ions (Fig. 1). The rapid initial binding may be due to the non-specific binding of ions to the external surface of the tissue, or it may represent ions which have diffused into the extrafibrous space of the attached inner shell membrane. The linear uptake of Mn^{2+} and Ca^{2+}, which followed the rapid initial binding, was saturable and could be described by Michaelis-Menten kinetics (Fig. 2). The apparent K_m for Mn^{2+} is almost identical to that for Ca^{2+}, and the V is slightly larger (Table III). It is significant that the kinetics of Ca^{2+} uptake over the first 2 min are similar to the kinetics of total Ca^{2+} transport over 3 h. The similarity of these values derived in the two experiments suggest that initial entry of divalent cations into the transporting cells is a major barrier in the transcellular transport process.

The kinetic studies also demonstrated that each ion would competitively inhibit the uptake of the other. This indicates that Mn^{2+} and Ca^{2+} share a common saturable pathway into the membrane. The values in Table III reveal that Mn^{2+} was more effective in blocking Ca^{2+} entry than Ca^{2+} was in blocking Mn^{2+} entry. This may be another indication that some Mn^{2+} is also entering the tissue passively.

The electron microprobe studies of the Mn^{2+} distribution in the chorioallantoic membrane showed Mn^{2+} in discrete localizations after exposure to Mn^{2+}. These Mn^{2+} concentrations were found only in the ectoderm of the tissue and in locations similar to those where Ca^{2+} concentrations have been found. The fact that Mn^{2+} and Ca^{2+} are both sequestered in high concentrations in the tissue is further evidence that they share a common pathway. Taken together, the experiments reported here strongly suggest that significant amounts of Mn^{2+} are taken up by the chorioallantoic membrane using the Ca^{2+} transport pathway. Consequently, EPR studies of Mn^{2+} incorporated into the tissue could provide further information on the intracellular mechanisms involved in the active transcellular transport of Ca^{2+} [11]. The chorioallantoic membrane is not unique in its handling of Mn^{2+}, since Mn^{2+} can substitute for Ca^{2+} in other biological systems. Mn^{2+} is accumulated by the Ca^{2+} transport system of mitochondria [15], and it inhibits Ca^{2+} accumulation by chick embryo fibroblasts [16] and human lymphocytes [17]. Mn^{2+} also affects muscle contraction [18] and neuromuscular transmission [19] by competing with Ca^{2+}. The ability of Mn^{2+} to substitute for Ca^{2+} in biological systems is probably due to the fact that they both have spherical charge distributions and that the 0.80 Å ionic radius of Mn^{2+} is close to the 0.98 Å radius of Ca^{2+}.

Although this study has emphasized the use of Mn^{2+} as an analog for Ca^{2+} the absorption of Mn^{2+} itself across epithelial tissues such as the intestine is an important biological problem. Mn^{2+} is a dietary requirement in birds and mammals,

and almost all of it is absorbed through the intestine [20]. In normal adult rats this Mn^{2+} uptake does not saturate [21], and there has been some uncertainty as to the effect of metabolic inhibitors on absorption [22, 23]. In iron-deficient rats Mn^{2+} has an affinity for the carrier-mediated iron absorption system of the intestine [24]. The techniques used here to study Mn^{2+} transport in the chorioallantoic membrane may also be useful in studying Mn^{2+} interaction with the calcium and iron transport mechanisms of the intestine.

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