Kinetic properties of the Ca^{2+} -accumulation system of a rat liver microsomal fraction

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1. By using Ca-EGTA buffers, the K_m for Ca^{2+} uptake into rat liver heavy microsomes (microsomal fraction) was found to be $0.2\ \mu\text{M}$ free Ca^{2+} . 2. In the absence of oxalate, these vesicles accumulate about $20\ \text{nmol}$ of Ca^{2+}/mg of protein. Efflux of Ca^{2+} from the vesicles is much faster at pH 7.6 than at pH 6.8, but does not apparently show saturation kinetics or any stringent requirement for external ions. 3. The steady-state distribution of Ca^{2+} between the microsomes and the medium in the presence of ATP and the absence of oxalate is dependent on Ca^{2+} load. When the vesicles are loaded to 50% capacity, the external free Ca^{2+} concentration is $70\ \text{nM}$. 4. The affinity of heavy microsomes for Ca^{2+} is such that it seems likely that they have a dominant role in the determination of cytoplasmic free Ca^{2+} concentrations.

The control of the cytoplasmic concentration of free Ca^{2+} is thought to be of great importance in the regulation of many cellular processes. In the particular case of hepatocytes, an increase in cytoplasmic free Ca^{2+} in response to α -adrenergic stimulation seems to be the most probable mechanism for the mediation of the stimulation of glycogenolysis by α -adrenergic agonists (Chen *et al.*, 1978; Blackmore *et al.*, 1978; Exton, 1979; Murphy *et al.*, 1980). Since α -agonists cause a transient efflux of Ca^{2+} from hepatocytes or perfused liver (Friedmann & Park, 1968; Chen *et al.*, 1978; Althaus-Salzmänn *et al.*, 1980), it is most likely that the increase in cytoplasmic Ca^{2+} arises from the mobilization of an intracellular store rather than from an inflow of extracellular Ca^{2+} . However, the nature of this store is in some doubt. Some results (Chen *et al.*, 1978; Blackmore *et al.*, 1979; Murphy *et al.*, 1980) are consistent with the mobilizable Ca^{2+} store being mitochondrial, since α -adrenergic stimulation leads to a decrease in Ca^{2+} content of a subsequently isolated mitochondrial fraction. Techniques where Ca^{2+} movements are followed by using $^{45}\text{Ca}^{2+}$, however, indicate that the initial effect of α -adrenergic stimulation is to enhance the incorporation of $^{45}\text{Ca}^{2+}$ into liver mitochondria (Althaus-Salzmänn *et al.*, 1980; Poggioli *et al.*, 1980; Berthon *et al.*, 1981), leading to the suggestion that the initial release of Ca^{2+} takes place from another site, which is possibly endoplasmic reticulum (Berthon *et al.*, 1981) or a pool of Ca^{2+} closely associated with the cell membrane (Althaus-Salzmänn *et al.*, 1980).

Isolated liver mitochondria buffer external Ca^{2+} at

approx. $1\ \mu\text{M}$ (Nicholls, 1978), which is somewhat above the concentration of cytoplasmic Ca^{2+} found by Murphy *et al.* (1980). Moreover, it has been suggested (Denton & McCormack, 1980) that the role of the mitochondrial Ca^{2+} -cycling system may be to modulate the activity of enzymes in the mitochondrial matrix, which would seem to be incompatible with a role in control of cytoplasmic Ca^{2+} as well. Becker *et al.* (1980) showed that a microsomal fraction from rat liver could lower external free Ca^{2+} concentrations to values similar to those found by Murphy *et al.* (1980), although their capacity for Ca^{2+} accumulation was rather small. It was found that microsomes (microsomal fraction), together with mitochondria to act as a high-capacity backing pump, behaved in a manner comparable with that of hepatocytes made permeable to Ca^{2+} by digitonin treatment (Becker *et al.*, 1980).

Both mitochondria and microsomes from rat liver have been shown to alter their Ca^{2+} -pumping activity in a stable way in response to various hormone treatments (Hughes & Barritt, 1978; Taylor *et al.*, 1979, 1980; Friedmann & Johnson, 1980; Andia-Waltenbaugh *et al.*, 1980; Reinhart & Bygrave, 1981). However, hormonally sensitive Ca^{2+} transport has been localized in the 'heavy' microsomal fraction rather than in the 'light' fraction (Taylor *et al.*, 1979). Previous studies on the kinetic properties of the Ca^{2+} -uptake system of microsomes (Moore *et al.*, 1975; Bygrave, 1978; Andia-Waltenbaugh *et al.*, 1980) have concentrated on the fraction sedimenting between $12\ 500\ \text{g}$ for 20 min and $105\ 000\ \text{g}$ for 60 min (Moore *et al.*, 1975). The

'heavy' microsomal fraction isolated by the method of Reinhart & Bygrave (1981), sedimenting between 7700 *g* for 10 min and 35 000 *g* for 20 min, has not been investigated in the same way. The present paper reports some observations on the kinetics of Ca^{2+} movements across the membranes of vesicles sedimenting in the heavy fraction. Vesicles of this type have a quite reasonable capacity for Ca^{2+} uptake (up to about 20 nmol of Ca^{2+} /mg of protein) in the absence of a precipitating anion such as oxalate. It has therefore been possible to measure rates of efflux of Ca^{2+} from preloaded microsomes and also to measure steady-state distribution of Ca^{2+} between the medium and the vesicle interior. The findings are consistent with a role for this Ca^{2+} -sequestering system in the maintenance of cytosolic Ca^{2+} concentrations.

Experimental

Materials

$^{45}\text{CaCl}_2$ (650 Ci/mol) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K., Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] was from Hopkin and Williams, and Ruthenium Red was from BDH, Poole, Dorset, U.K. The Ruthenium Red was purified by the method of Fletcher *et al.* (1961) and its concentration was determined by measurement of A_{532} , by using an A_{mM} of 61.5 (Fletcher *et al.*, 1961). For experiments where it was necessary to minimize endogenous free Ca^{2+} , experimental media, before the addition of any bivalent metal ions or chelating agents, were passed through a column of Chelex 100 and stored in plastic containers. The Ca^{2+} content of solutions treated in this way was typically $<2\mu\text{M}$, as measured by atomic-absorption spectrophotometry.

Preparation of microsomes

Microsomes were prepared from the livers of fed 200–250 g male albino Wistar rats, essentially as described by Reinhart & Bygrave (1981). The livers were chopped and homogenized (10%, w/v) in 250 mM-sucrose/5 mM-Hepes/KOH (pH 6.8)/1 mM-dithiothreitol/0.5 mM-EGTA, with five strokes of a Dounce homogenizer (0.2 mm radial clearance). The homogenate was centrifuged at 1000 *g* for 5 min and the supernatant was re-centrifuged at 8000 *g* for 10 min. Microsomes were then prepared from the resulting supernatant by centrifugation at 35 000 *g* for 20 min. The pellet was resuspended in 0.5 vol (relative to the initial homogenate) of 250 mM-sucrose/5 mM-Hepes/KOH (pH 6.8)/10 mM-KCl/1 mM-dithiothreitol and centrifuged again at 35 000 *g* for 20 min. The pellet was resuspended in a small volume of the same buffer to give a protein concentration of 20–35 mg/ml. Specific activities of glucose 6-phosphatase and cytochrome oxidase were

similar to those reported by Reinhart & Bygrave (1981) for this fraction.

Protein determination

This was done by the method of Lowry *et al.* (1951) after clarification with sodium dodecyl sulphate. Bovine serum albumin (Sigma fraction V) was used as a standard.

Ca^{2+} -uptake assays

These were done by a modification of the filtration methods used by Taylor *et al.* (1979) and Moore *et al.* (1975). The temperature was 37°C. The incubation mixture contained, in a total volume of 1 ml: 100 mM-KCl, 20 mM-Hepes/KOH buffer (pH 6.8), 5 mM-MgCl₂, 5 mM-ammonium oxalate, 1 mM-dithiothreitol, 3 μM -Ruthenium Red, the required concentration of Ca^{2+} or Ca^{2+} -EGTA buffer, 0.5 μCi of $^{45}\text{Ca}^{2+}$ and 0.6–1.0 mg of microsomal protein. After preincubation for 5 min at 37°C, uptake was started by the addition of 2.5 mM-ATP. At the required time intervals after ATP addition, 0.1 ml samples were removed and filtered rapidly through Millipore disc filters (0.45 μm pore size) prewetted with 100 mM/KCl/20 mM-Hepes/KOH (pH 6.8)/5 mM-MgCl₂. The filters were washed rapidly with 2 $\times$ 1 ml of this same buffer at 4°C, and dropped into scintillation vials containing 3 ml of scintillant (4.67 g of 2,5-diphenyloxazole in 660 ml of toluene and 340 ml of Triton X-100). Counts were recorded on the ^{14}C channel of a Philips 4700 scintillation counter, with an efficiency of 80%.

Ca^{2+} -efflux assays

Microsomes were first loaded with Ca^{2+} by incubation at 37°C in a medium containing, in a volume of 1 ml, 100 mM-KCl, 20 mM-Hepes/KOH (pH 6.8), 5 mM-MgCl₂, 1 mM-dithiothreitol, 2 μCi of $^{45}\text{Ca}^{2+}$, 3 μM -Ruthenium Red, 4–6 mg of microsomal protein, 2.5 mM-ATP and the required concentration of unlabelled CaCl_2 . After 5 min, the mixture was centrifuged for 2.5 min in an Eppendorf 3200 centrifuge (15 000 *g*) and the pellet rapidly resuspended in 0.5 ml of 100 mM-KCl/20 mM-Hepes/KOH (pH 6.8)/5 mM-MgCl₂/1 mM-dithiothreitol pre-cooled to 0°C. Then 0.1 ml of the resuspended pellet was immediately transferred to 0.9 ml of the efflux medium (defined in the Figure legends) pre-equilibrated at 37°C, and 0.1 ml samples of this were then removed at suitable time intervals and filtered by the same procedure as described above. Samples of the resuspended pellet were taken for measurements of total radioactivity by scintillation counting and, after precipitation by trichloroacetic acid, total Ca^{2+} by atomic-absorption spectrophotometry. Extrapolation of the ^{45}Ca retained on the filter to zero time thus allowed the calculation of the initial Ca^{2+} loading of the microsomes, assuming that the initial

distribution of ^{45}Ca reflected the initial distribution of total Ca^{2+} .

Determination of free Ca^{2+} concentration

Free Ca^{2+} concentrations in media containing several interacting ligands (Ca^{2+} , Mg^{2+} , H^+ , oxalate, ATP, and EGTA) were initially calculated by using a computer program kindly run by Dr. J. C. Hutton, Department of Clinical Biochemistry, Cambridge, on the University of Cambridge Phoenix computer. The program was based on the method described by Storer & Cornish-Bowden (1976). The same program was later adapted by Dr. J. A. Bangham of our Department for use on an Apple II micro-computer. Association constants were taken from Sillen & Martell (1964).

Results

Ca^{2+} uptake

Moore *et al.* (1975) found that the pH optimum for Ca^{2+} accumulation by 'light' microsomes was about pH 6.8. The same is true for the fraction of microsomes used in the present study (Fig. 1) as long as Ca^{2+} uptake is measured after 90 s. At shorter times, the optimum pH shifts to a more alkaline value, particularly in the presence of the

Fig. 1. Microsomal Ca^{2+} uptake as a function of pH. The ^{45}Ca -uptake medium was as described in the Experimental section and was adjusted to the required pH with KOH. Microsomal protein concentration was 0.68 mg/ml and total Ca^{2+} concentration was 50 μM . Uptake was started by the addition of ATP (adjusted to the required pH) and samples were removed at 30 s (○, ●) and 90 s (△, ▲). ○, △, No oxalate present; ●, ▲, plus 5 mM-ammonium oxalate. Values for Ca^{2+} accumulation are corrected for Ca^{2+} binding in the absence of ATP.

precipitating anion oxalate. This suggests that changing the pH might be having an effect on the shape of the progress curve for Ca^{2+} uptake, which was found by Bygrave (1978) and Taylor *et al.* (1979) to be biphasic for the light-microsomal fraction. Fig. 2 shows progress curves for Ca^{2+} uptake at pH 6.8 and pH 7.6 in the presence and absence of oxalate. In the presence of oxalate at pH 6.8 there is an initial rapid phase of Ca^{2+} uptake, complete within about 2 min, followed by a linear slower phase which continues for a long period. The omission of oxalate has essentially no effect on the initial rapid phase of Ca^{2+} uptake, in contrast with the findings of Bygrave (1978). However, in agreement with the experiments of Bygrave (1978), in the absence of oxalate, uptake ceases after a relatively short time (4 min) and after that time the partition of Ca^{2+} between the medium and the microsomes remains constant. Increasing the pH to 7.6 decreases the extent of the initial rapid phase of uptake, but has relatively little effect on the initial velocity, as suggested by the data in Fig. 1. Increasing the pH also abolishes the slow linear accumulation phase observed in the presence of oxalate. These results strongly suggest that, as for 'light' microsomes (Taylor *et al.*, 1979), the slow phase of Ca^{2+} uptake seen in the presence of oxalate is accompanied by Ca^{2+} recycling. It is therefore essential in studies of the kinetics of Ca^{2+} uptake to use initial-rate measurements as much as possible.

The dependence of the rate of Ca^{2+} uptake on Ca^{2+} concentration has given rather variable results for light microsomes. Moore *et al.* (1975) reported a value of 23 μM - Ca^{2+} (total) for K_m for Ca^{2+} uptake, corresponding to a Ca^{2+} (free) concentration of about 4 μM . A similar value was reported by Andia-Waltenbaugh *et al.* (1980). However, Bygrave (1978), using initial-rate measurements, found that K_m was about 1–2 μM - Ca^{2+} (total), suggesting a considerably lower value for Ca^{2+} (free). Fig. 3(a) shows the dependence of initial rate of Ca^{2+} uptake on free Ca^{2+} concentration for heavy microsomes, where the initial free Ca^{2+} concentration was stabilized by the use of Ca^{2+} -EGTA buffers. The double-reciprocal plot (Fig. 3b) is linear and gives a value for K_m of 0.2 μM -free Ca^{2+} , and V_{max} = 16 nmol/min per mg.

Ca^{2+} efflux

The steady-state accumulation observed in the absence of oxalate (Fig. 2) is likely to be determined by the balance of uptake rate against an efflux process, as is the case for mitochondria (Nicholls, 1978). Since the initial rate of Ca^{2+} uptake is not greatly changed by altering the pH from 6.8 to 7.6, it seems probable that the dominant effect responsible for the 4-fold decrease in steady-state accumulation between these two pH values is an increase in efflux

Fig. 2. Time course of Ca^{2+} accumulation

Conditions were as for Fig. 1. (a) No oxalate present; (b) 5 mM-ammonium oxalate present: ○, pH 6.8; ●, pH 7.6.

Fig. 3. Dependence of initial velocity of Ca^{2+} accumulation on free Ca^{2+} concentration

Ca^{2+} uptake was measured as described in the Experimental section. The pH of the medium was 6.8. The incubation mixture contained 5 mM-ammonium oxalate, 0.6 mg of microsomal protein/ml and 62 μM total Ca^{2+} . The indicated initial free Ca^{2+} concentrations were obtained by the addition of 0, 62.5, 188, 312, 438, 562 and 625 μM-EGTA. Ca^{2+} accumulation was measured 1 min after the addition of ATP.

rate. This is confirmed by the observations shown in Fig. 4. Microsomes loaded under standard conditions at pH 6.8 were diluted into KCl-based media adjusted to the required pH. It is clear that the rate of leakage of $^{45}\text{Ca}^{2+}$ from the microsomes increases dramatically with pH.

In terms of a possible role for microsomes in control of cytoplasmic Ca^{2+} concentrations, efflux of Ca^{2+} from intracellular storage is likely to be of

Fig. 4. Efflux of Ca^{2+} from microsomes
Microsomes were loaded with $^{45}\text{Ca}^{2+}$ under the conditions described in the Experimental section. The total Ca^{2+} concentration was 70 μM and the microsomal concentration was 6 mg/ml. The initial Ca^{2+} loading was 10 nmol/mg. The efflux media contained 100 mM-KCl, 5 mM-MgCl₂, 1 mM-dithiothreitol and 20 mM-Hepes adjusted with KOH to: ●, pH 6.5; ○, pH 6.8; △, pH 7.2; ▲, pH 7.6.

equal importance to the uptake process. However, the data shown in Fig. 4 give no information on whether the increased efflux rate observed at alkaline pH reflects the activity of a specific Ca^{2+} -conducting pathway or an increase in non-specific permeability of the microsomal membrane. It was therefore decided to investigate Ca^{2+} efflux in rather more detail. Fig. 5 shows efflux rate as a function of initial Ca^{2+} loading of the microsomes. There appears to be a linear relationship between loading and initial rate of efflux, so that if the efflux process can be saturated it is not possible to get any evidence of this, given the experimental limitation of an initial maximum Ca^{2+} loading of about 20 nmol/mg of protein (Fig. 2). It appears also (Fig. 6) that there is no very specific external ionic requirement for Ca^{2+} efflux. Efflux is somewhat faster in a NaCl-based medium than in KCl, and in both these media the rate is considerably faster than when sucrose provides the osmotic support. Replacement of Cl^- by isethionate has no effect (results not shown). The rate of release of an internal species will depend on the degree of osmotic support provided by the external medium (Meissner & Allen, 1981), and differences in efflux rate may reflect differences in permeability of the microsomal membrane to NaCl, KCl and sucrose, rather than direct effects on Ca^{2+} transport. Efflux of Ca^{2+} in these experiments is not affected by the addition of EGTA, suggesting that

Fig. 5. *Dependence of velocity of efflux on Ca^{2+} loading* Microsomes (5 mg of protein/ml) were loaded in media containing from 0 to 500 μM added Ca^{2+} to give the initial loadings shown. The efflux medium contained 100 mM-KCl, 1 mM-dithiothreitol, 5 mM- MgCl_2 and 20 mM-Hepes adjusted to pH 7.6 with KOH. Initial Ca^{2+} loading was measured as described in the Experimental section, and the initial velocity of efflux was determined from the decrease in microsomal Ca^{2+} content between 15 and 60 s after initiation of efflux.

Fig. 6. *Effect of composition of the external medium on the efflux velocity*

Microsomes (6 mg of protein/ml) were loaded as described in the legend to Fig. 4. The efflux media contained 5 mM- MgCl_2 , 1 mM-dithiothreitol and: O, 100 mM-KCl 20 mM-Hepes/KOH, pH 7.6; ●, 100 mM-NaCl, 20 mM-Hepes/NaOH, pH 7.6; Δ, 250 mM-sucrose, 20 mM-Hepes/Tris, pH 7.6.

external free Ca^{2+} does not inhibit outward diffusion of Ca^{2+} . There is thus no positive evidence as yet to indicate that the efflux observed under these experimental conditions reflects the activity of a specific Ca^{2+} efflux route, rather than a general change in ionic permeability of the membrane.

Steady-state Ca^{2+} distribution

The steady Ca^{2+} accumulation observed in the absence of oxalate but the presence of ATP (Fig. 2) suggested that microsomes might behave as Ca^{2+} buffers, a view supported by the observations of Becker *et al.* (1980). The experiment shown in Fig. 7 was designed to examine this behaviour. Microsomes, in the absence of oxalate, were allowed to accumulate Ca^{2+} until a steady state was reached. The loading obtained was altered by varying the total Ca^{2+} concentration at a series of fixed EGTA concentrations. From the distribution of $^{45}\text{Ca}^{2+}$ between the microsomes and the medium, and the total Ca^{2+} present in the incubation mixture, the Ca^{2+} contents of the microsomes and the external medium were calculated. The free Ca^{2+} in the external medium was then obtained by using the program described in the Experimental section. Given the experimental limitations imposed by endogenous Ca^{2+} and the requirement, for accurate data, of a significant percentage of the total Ca^{2+} to be accumulated by the microsomes, it was not possible to do the experiment at a single EGTA

Fig. 7. Steady-state Ca^{2+} loading as a function of external free Ca^{2+} concentration

Microsomes (1 mg of protein/ml) were suspended in a medium containing 100 mM-KCl, 20 mM-Hepes/KOH, pH 6.8 (O, ●, Δ) or 7.6 (▲), 5 mM- MgCl_2 , 0.15 μCi of ^{45}Ca /ml, 0.5 μCi of $^3\text{H}_2\text{O}$ /ml, 1 mM-dithiothreitol, 1.5 μM -Ruthenium Red and 18 μM -EGTA (Δ, ▲), 36 μM -EGTA (O) or 136 μM -EGTA (●). Total Ca^{2+} concentration was adjusted over the range 10–30 μM for each EGTA concentration. After 5 min at 37°C, uptake was started by the addition of 2.5 mM-ATP, and 1 ml samples were removed after 5 and 8 min reaction time. The samples were immediately centrifuged for 2.5 min at 15000 g. Pellet and supernatant were counted for ^{45}Ca and ^3H radioactivities, and ^{45}Ca in the pellet was corrected for ^{45}Ca in the aqueous space. Ca^{2+} distribution was calculated from the isotopic distribution and total Ca^{2+} content measured, after precipitation with trichloroacetic acid, by atomic absorption. There was no significant difference between the Ca^{2+} distributions at 5 and 8 min, and the data shown are the average of the two.

concentration. However, it is clear that the regions of the curve obtained at different EGTA concentrations overlap precisely, and there is no indication of any discontinuities. For this reason it seems very unlikely that there is a direct effect of EGTA on the accumulation system. As would be expected from the K_m value obtained for Ca^{2+} uptake and the rather low efflux rate from loaded microsomes at pH 6.8, the effective affinity of microsomes for Ca^{2+} in the presence of ATP is very high. When the microsomes are loaded to 50% of their capacity, the external steady-state free Ca^{2+} concentration is about 70 nM. At pH 7.6, the maximum loading capacity is greatly decreased, but the system is still capable of lowering the external free Ca^{2+} concentration to at least 0.1 μM .

Discussion

The kinetic behaviour *in vitro* of the Ca^{2+} -uptake system of rat liver microsomes appears to be

consistent with a role for this subcellular fraction in the control of the cytoplasmic Ca^{2+} concentration. The K_m for Ca^{2+} uptake is similar to the values for free cytoplasmic Ca^{2+} concentration reported by Murphy *et al.* (1980), and the microsomes are capable of lowering the external Ca^{2+} concentration to values below 0.1 μM . In terms of their properties *in vitro* it seems very likely that microsomes would compete successfully with mitochondria for uptake of Ca^{2+} in the cell. Their ability to buffer external Ca^{2+} concentration is, however, rather different in nature from that of mitochondria. Because the rate of Ca^{2+} efflux from liver mitochondria is independent of loading over quite a wide range (Dawson & Fulton, 1980), mitochondria act as 'absolute' Ca^{2+} buffers, i.e. the external steady-state Ca^{2+} concentration is independent of loading (Nicholls, 1978). However, efflux from microsomes under the experimental conditions used here is proportional to the internal Ca^{2+} load, with the result that the buffering characteristics of microsomes are more analogous to those of a conventional Ca^{2+} -chelating agent.

Although from the above considerations it is a reasonable assumption that microsomes play an important role in maintaining cytoplasmic Ca^{2+} concentrations, it is not yet clear what this role might be. If the origin of the vesicles responsible for Ca^{2+} uptake is intracellular, then it is likely that they act as intracellular Ca^{2+} stores, as envisaged by Berthon *et al.* (1981). Hales *et al.* (1974) showed that the smooth endoplasmic reticulum in rat adipocytes contained large amounts of Ca^{2+} *in situ* and suggested that it might be analogous, structurally and functionally, to sarcoplasmic reticulum. If this is the case for hepatocytes as well, then, in addition to a Ca^{2+} -accumulation system, there should be some form of release mechanism which can be triggered to mobilize internalized Ca^{2+} . The efflux process examined in the present paper seems to be rather slow at pH 6.8, and there is no evidence as yet to suggest that the faster efflux at pH 7.6 represents a specific Ca^{2+} -release process. Efflux of Ca^{2+} from sarcoplasmic reticulum is also enhanced by increasing the pH above neutrality (Duggan & Martonosi, 1970). In this latter case it has been suggested that Ca^{2+} release is controlled by a proton gradient across the sarcoplasmic-reticulum membrane (Shoshan *et al.*, 1981). In view of the inhibition of Ca^{2+} uptake into rat liver microsomes by uncouplers (Bygrave, 1978), it is clearly a possibility that a similar system operates in these vesicles.

An alternative view of the microsomal Ca^{2+} -transport system is that the vesicles concerned arise from the plasma membrane, and that topologically the inside of the vesicle is extracellular. In this case, Ca^{2+} transport would presumably be a manifestation of the outward Ca^{2+} pump observed in liver

slices (Cittadini & Van Rossum, 1978). Although the fraction containing Ca²⁺-transport activity is enriched in glucose 6-phosphatase activity, 5'-nucleotidase activity is also considerable (Reinhart & Bygrave, 1981), so that an origin of this sort cannot yet be ruled out. In view of the stimulation of Ca²⁺ uptake by glucagon observed for these vesicles (Reinhart & Bygrave, 1981), it is clearly of importance to establish their precise cytological origin.

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