Microsomal Membrane Permeability and the Hepatic Glucose-6-phosphatase System

INTERACTIONS OF THE SYSTEM WITH D-MANNOSE 6-PHOSPHATE AND D-MANNOSE*

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We have proposed that glucose-6-phosphatase (EC 3.1.3.9) is a two-component system consisting of (a) a glucose-6-P-specific transporter which mediates the movement of the hexose phosphate from the cytosol to the lumen of the endoplasmic reticulum (or cisternae of the isolated microsomal vesicle), and (b) a nonspecific phosphohydrolase-phosphotransferase localized on the luminal surface of the membrane (Arion, W. J., Wallin, B. K., Lange, A. J., and Ballas, L. M. (1975) Mol. Cell. Biochem. 6, 75-83). Additional support for this model has been obtained by studying the interactions of D-mannose-6-P and D-mannose with the enzyme of untreated (i.e. intact) and taurocholate-disrupted microsomes.

An exact correspondence was shown between the mannose-6-P phosphohydrolase activity at low substrate concentrations and the permeability of the microsomal membrane to EDTA. The state of intactness of the membrane influenced the kinetics of mannose inhibition of glucose-6-P hydrolysis; uncompetitive and noncompetitive inhibitions were observed for intact and disrupted microsomes, respectively. The apparent $K_a$ for glucose-6-P was smaller with intact preparations at mannose concentrations above 0.3 M. Mannose significantly inhibited total glucose-6-P utilization by intact microsomes, whereas D-glucose had a stimulatory effect. Both hexoses markedly enhanced the rate of glucose-6-P utilization by disrupted microsomes.

The actions of mannose on the glucose-6-phosphatase of intact microsomes fully support the postulated transport model. They are predictable consequences of the synthesis and accumulation of mannose-6-P in the cisternae of microsomal vesicles which possess a nonspecific, multifunctional enzyme on the inner surface and a limiting membrane permeable to D-glucose, D-mannose, glucose-6-P, but impermeable to mannose-6-P.

The latency of the mannose-6-P phosphohydrolase activity is proposed as a reliable, quantitative index of microsomal membrane integrity. The inherent limitations of the use of EDTA permeability for this purpose are discussed.

Liver and kidney microsomal glucose-6-phosphatase (EC 3.1.3.9) catalyze the terminal reactions of gluconeogenesis and glycogenolysis. Among the enzymes constituting the glucogenic pathways in liver and kidney, glucose-6-phosphatase is unique by virtue of its intimate association with the membranous elements of the endoplasmic reticulum. Recent efforts in this laboratory have been directed toward the elucidation of the molecular basis and physiologic significance of this association. The results of our studies led to the proposal (1) that glucose-6-phosphatase is a two-component system consisting of (a) a glucose-6-P transporter which mediates the transfer of glucose-6-P across the membrane of the endoplasmic reticulum or microsome, and (b) a nonspecific phosphohydrolase-phosphotransferase localized on the cisternal aspect of the membrane.

This concept of glucose-6-phosphatase, designated herein as the substrate transport hypothesis or transport model, is based on two related propositions: (a) substrate utilization occurs subsequent to its penetration through the permeability barrier into the cisternal space where the catalytic component (i.e. the intrinsic enzyme) is located; and (b) an intramicrosomal pool of glucose-6-P exists. Thus, the feature of selective permeability of the microsomal membrane was incorporated into the model to explain the high degree of specificity of the enzyme of untreated (i.e. intact) microsomes for glucose-6-P (2), and the involvement of a glucose-6-P-specific transport system was postulated to provide the intrinsic enzyme access to glucose-6-P. An important corollary is that alternate substrates for the catalytic component, such as carbamoyl-P and mannose-6-P (3), are poorly utilized by intact microsomes because they are only weakly recognized by the transporter or otherwise
unable to penetrate the membrane barrier. Thus, the associated phosphohydrolase and phosphotransferase activities remain unexpressed (e.g., latent) until the catalytic component is rendered free access to the alternate substrates by treatments which destroy the microsomal permeability barrier (e.g., exposures of microsomes to extremes of pH (2, 4), various detergents (5, 6), nitrogen cavitation (7), or ultrasound).

The validity of the foregoing propositions was evaluated in the experiments described in this report in which the interactions of the glucose-6-phosphatase system with D-mannose and D-mannose-6-P were studied. The results of this investigation corroborate predictions derived from theoretical considerations of the transport model.

EXPERIMENTAL PROCEDURE

Enzyme Preparations—Male rats of Long-Evans descent, weighing approximately 160 g, were obtained from Blue Spruce Farms (Al-
tamont, N.Y.) and maintained on Charles Rivers laboratory chow and water, ad libitum, for 2 weeks. Liver microsomes were prepared as described previously (8). Liver microsomes from 24-hour fasted rats were obtained in all experiments except those summarized in Tables I and II, where fed animals were employed. "Intact microsomes" are microsomes which were diluted to the desired concentration with 0.25 M sucrose/5 mM Tris acetate (pH 7.4) and assayed without further treatment. "Disrupted microsomes" were prepared at 0°C by supplementing 9 volumes of intact microsomes (1 mg of protein/ml) with 1 volume of 4% sodium taurocholate, pH 7.8. The taurocholate-dis-
persed microsomes were kept on ice at least 30 min before assays were performed.

Materials—The barium salts of glucose-6-32P and mannose-6-32P were prepared by previously described procedures (2) with some modifications: (a) bovine heart mitochondria (9), twice washed by centrifugation in 0.28 M mannitol, were used in place of the submicro-
chondrial particles, and (b) the final concentration of carrier P2 in the incubation media was reduced to 1 mM. These changes permitted the incorporation of as much as 90% of the total 32P into the hexose phosphates in one-fifth the time required by the original procedure, and the specific radioactivities of the hexose [32P]phosphates were 10 times greater. The barium salt of D-[U-14]C]hexose-6-32P was prepared as described earlier (10). D-[U-14C]Hexoses and [32P] were obtained from the International Chemical and Nuclear Corp. [14C]Hexoses were purified further (10). Aqueous solutions of the barium salts of the hexose 6-phosphates were converted to sodium salts by passage through columns of Amberlite AG 50xW-X4 (Bio-Rad) and adjustment of the effluent to pH 6.5 with dilute NaOH. Sources or methods of preparation of other chemicals were as described earlier (2, 6, 10).

Analytical Methods—Protein was determined by the biuret procedure (11) using crystalline bovine plasma albumin as the reference standard. Incubations with enzyme were for 10 min at 30°C, except as noted (see Fig. 3). Assay media are described in the legends to tables and figures. Except for the experiment described in Fig. 3, microsomes are equally sensitive to inhibition by glucose-6-P. The simplest explanation for the biphasic kinetic behavior depicted in Fig. 1A is that microsomes contain two mannose-6-P phosphohydrolases with disparate Michaelis constants. Several lines of evidence indicate that the catalytic component of the glucose-6-phosphatase system in disrupted microsomes catalyzes the "low K" activity. First, the Michaelis constants for mannose-6-P, evaluated from Curve I in Fig. 1A and from either of the lines in Fig. 1B, are essentially identical (see legend to Fig. 1). Second, it was shown earlier (2) that at low substrate concentrations the mannose-6-P phosphohydrolase activities of untreated and detergent-disrupted microsomes are equally sensitive to inhibition by glucose-6-P.

Last, the mannose-6-P phosphohydrolase activity at low sub-
strate concentrations is unaffected by sulfhydryl poisons, as is the glucose-6-P phosphohydrolase activity of disrupted, but not intact, microsomes.

RESULTS AND DISCUSSION

Mannose 6-P Phosphohydrolase Activities of Intact and Disrupted Microsomes—In an earlier study (2) we observed that the mannose-6-P phosphohydrolase activity of untreated rat liver microsomes was activated by elevating the concentration of mannose-6-P. This unexplained behavior was further scrutinized in the present study. The influence of substrate concentration on the rates of mannose-6-P hydrolysis catalyzed before and after supplementation of microsomes to 0.4% sodium taurocholate are compared in the double reciprocal plots presented in Fig. 1A and B. The data for detergent-
treated microsomes (Fig. 1B) define a straight line only over the range 0.15 to 20 mM. At higher concentrations (28 to 50 mM, Curve II) there was some inhibition by mannose-6-P. Data for untreated microsomes (Fig. 1A) define a straight line only at low concentrations (0.15 to 1.5 mM, Curve I), whereas at higher levels of mannose-6-P (Curve II) "activation" occurred.

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strate concentrations is unaffected by sulfhydryl poisons, as is the glucose-6-P phosphohydrolase activity of disrupted, but not intact, microsomes.

Fig. 2 shows that the biphasic kinetic data in Fig. 1A can be resolved to yield the kinetic characteristics of the "low K".

- Inhibition of glucose-6-P hydrolysis by glucose-6-P concentrations above 50 mM is seen in the data of Berteloot et al. (14). From considerations of the reaction mechanism (15, and see below), substrate inhibition probably arises at these high concentrations from binding of the hexose moieties of these substrates to the phosphoryl acceptor site (i.e., site of water or hexose binding) with the resultant formation of an unreactive phosphoryl enzyme-hexose phosphate complex.

- Because of the broad specificity and multifunctional nature of the catalytic component of the glucose-6-phosphatase system (3, 15), it is necessary to clearly identify the reaction in which a kinetic constant refers. We have adopted the following notation: Subscripts "h" and "t" are added to Michaelis constants (K) and maximal velocities (V) to denote phosphohydrolase and phosphotransferase, respectively. Phosphoryl donor substrates are identified by a second subscript in parentheses. The abbreviations used are: G6P, glucose-6-P; M6P, mannose 6-P; Man, d mannose.
assays of disrupted or intact microsomes. x axes I and II define
and after (B) supplementation of microsomes to 0.4% sodium tauro-
clude. Data points on Curve I or II (see legend to Fig. 2) are plotted
and maximal velocities3 (units per mg of protein), evaluated as re-
ciprocals of x and y axis intercepts were from Curve I, A, Kd(MAP) =
51 mM and Vmax = 0.02; from Curve I and II, B, Kd(MAP) = 0.71
mM and Vmax = -0.39.

activity. This was accomplished by subtracting the contribu-
tion of the low Km activity from the total activity at the higher
concentrations of substrate (see legend to Fig. 2). In the double
reciprocal plot, the calculated data points clearly define a
straight line (y = 773x + 15.2; coefficient of determination, r²,
equal to 1.00). Therefore, the kinetic data in Fig. 1A conform to
that expected for a system possessing two mannose-6-P phos-
phohydrolase activities of markedly different Michaelis con-
stants, namely 0.71 and 51 mM. The exactness with which the
data fit this explanation tends to rule out alternative interpre-
tations for the "substrate activation." For example, it is
unlikely that the kinetic behavior is the expression of a single
enzyme exhibiting cooperative (allosteric) behavior. The possi-

FIG. 1. Kinetics of mannose-6-P hydrolysis catalyzed before (A)
and after (B) supplementation of microsomes to 0.4% sodium tauro-
cholate. Assay mixtures (pH 6.5) contained the following in a final
volume of 1.0 ml: 50 mM Tris-cacodylate buffer, 10 mg of bovine
plasma albumin, concentrations of mannose-6-P between 0.15 and
50 mM, and 0.1 or 0.2 mg of microsomal protein, respectively, for
assays of disrupted or intact microsomes. x axes I and II define

FIG. 2. Kinetics of the high Km mannose-6-P phosphohydrolase of
untreated microsomes. Initial rates of the high Km activity were
estimated at these high substrate concentrations by normalizing the values plotted in Curve II,
activity in viuo. However, a comparison of the kinetic constants for
mannose-6-P and glucose-6-P hydrolysis by intact microsomes
activity in viuo. However, a comparison of the kinetic constants for
mannose-6-P and glucose-6-P hydrolysis by intact microsomes
was considered; however, neither the low Km nor the high Km
activities were diminished by repeated washings of the mi-
below) indicated that the increase in activity at low substrate concentration was due to an increase
in the proportion of disrupted microsomes.

We believe the high Km mannose-6-P phosphohydrolase is
catalyzed by the glucose-6-phosphatase system of intact mi-
activity was usually observed. A second index of
membrane intactness (see below) indicated that the increase in
activity at low substrate concentration was due to an increase
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Fig. 3. Effects of taurocholate treatment on the activity of the low 
K_m of mannose-6-P phosphohydrolase (x-x) and the permeability of the 
microsomal membrane to EDTA (O-O). Lead [Pb(II)]-loaded microsomes were used to assess the permeability of EDTA (see 
"Experimental Procedure."), while untreated microsomes were 
employed in the phosphohydrolase assays. In both cases, 9 volumes of 
microsomes (1 mg/ml in buffered 0.25 M sucrose) were supplemented 
with 1 volume of a stock solution of sodium taurocholate, pH 7.6, to 
yield the indicated final concentration of detergent. All preparations 
were supplemented to 4 mM EDTA. Mannose-6-P phosphohydrolase 
activities were assayed at 0° by incubation of 0.9 ml of sample with 0.1 
ml of a solution containing 1 mM of mannose-6-P and 25 mM of 
Tri-acetate buffer, final pH of 6.5. Incubations were for 2 min. 
Other details are described under "Experimental Procedure." 

To achieve this correlation it was necessary to evaluate EDTA 
permeability and mannose-6-P phosphohydrolase activity under identical 
conditions. Our usual enzyme assay procedure is to add 0.1 ml of 
taurocholate-supplemented microsomes to 0.9 ml of assay medium, 
with the result that the detergent is diluted 10-fold. However, when 
microsomes were exposed to concentrations of taurocholate below 0.2% 
(w/v), detergent activation was partially reversed by dilution of the 
activated preparations prior to assay. Thus, when the usual enzymatic 
assay procedure was used in the experiment summarized in Fig. 3, the 
line describing the response of mannose-6-P phosphohydrolase was 
shifted to the right of that for EDTA penetration at concentrations of 
detergent between 0.04 and 0.16%. The problem of detergent dilution 
was minimized by adding 0.9 ml of taurocholate-treated microsomes to 
0.1 ml of assay medium and decreasing the incubation time and 
temperature. Under these conditions, inhibition of the phosphohydrolase 
by taurocholate (see Ref. 6) was negligible.
considerations of the substrate transport hypothesis, certain system was analyzed with regard to the effects of added actual experimental observations. The glucose-6-phosphatase nose-6-P can be predicted and, therefore, compared with mannose on (a) the kinetics of glucose-6-P hydrolysis, and (b) the rate of utilization of glucose-6-P.

Mannose Inhibition of Glucose-6-P Phosphohydrolase Activities of Intact and Disrupted Microsomes—Lineweaver-Burk plots (23) describing the inhibition by mannose are presented in Fig. 4. Apparent Michaelis constants and apparent maximal velocities were calculated from x and y axis intercepts, respectively, at each concentration of mannose. These values, along with values for the inhibitor constant for mannose (K
\text{man})
, are tabulated in Fig. 4. Values for the latter constant, equivalent to Cleland's K
\text{intercept}
, (24) were calculated fromordinate intercepts as described by Hass and Byrne (25). The data for disrupted microsomes (Fig. 4B) confirmed earlier findings (15). The earlier paper should be consulted for a discussion of the significance of the intersection point. With intact microsomes (Fig. 4A), increasing concentrations of mannose generated a family of parallel lines, reminiscent of uncompetitive inhibition. A comparison of Fig. 4, A and B, reveals that as the level of mannose was increased, the apparent Michaelis constants for intact and disrupted preparations approached each other, and at concentrations of mannose above 0.3 mM the apparent K
\text{intercept}
 of intact microsomes was actually smaller than the corresponding value for the detergent-treated preparation.

The finding of a smaller K
\text{intercept}
 for intact microsomes at high levels of mannose is in accord with predictions derived from theoretical considerations of the transport model (1). It is important to recall that mannose-6-P is essentially as good a substrate for the intrinsic enzyme as is glucose-6-P (cf. Figs. 1B and 4B, and Refs. 9 and 15). Consequently, the hydrolysis of mannose-6-P and glucose-6-P within the cisternae would be kinetically indistinguishable. Thus in the absence of mannose-6-P efflux, a functional glucose-6-P-mannose phosphotransferase would create the unique situation in which the catalytic component of intact microsomes is bathed in a medium containing a higher concentration of hydrolyzable substrate than that in contact with the enzyme of disrupted microsomes, i.e. the combined concentrations of glucose-6-P and mannose-6-P within the cisternae would exceed the level of glucose-6-P in the assay medium. One characteristic of this unique system would be the observation of a smaller apparent Michaelis constant for glucose-6-P in intact microsomes, when compared with the value for disrupted microsomes obtained under identical assay conditions.

Effects of Mannose on Rates of Utilization of Glucose-6-P by Intact and Disrupted Microsomes—A brief review of the reaction mechanism is required to understand the rationale for this study. The generally accepted mechanism for the glucose-6-P phosphohydrolase and glucose-6-P-hexose phosphotransferase activities involves the following sequence of reactions (3, 15): (a) formation of a binary complex between enzyme and glucose-6-P; (b) dissociation of glucose leaving a phosphoryl-enzyme intermediate; and (c) transfer of the phosphoryl group either to water (phosphohydrolase activity), glucose (glucose-6-P-glucose exchange activity), or other hexose (glucose-6-P-hexose phosphotransferase activity). Phosphotransfer to water is the slowest step in the hydrolysis of glucose-6-P, as evidenced by the finding that added [¹⁴C]glucose enforces the rate of formation of [¹⁴C]glucose from [¹⁴C]glucose-6-P (10, 15). It should be noted that the utilization of glucose-6-P in both the phosphotransferase and phosphohydrolase reactions (i.e. total glucose-6-P utilization) may be evaluated by determining the formation of [¹⁴C]glucose from labeled glucose-6-P, whereas assays for P, assess only that fraction of glucose-6-P undergoing hydrolysis.

The effects of D-glucose and D-mannose on the rate of utilization of glucose-6-P by intact and disrupted microsomes are compared in Table I. The addition of glucose to intact microsomes or the presence of mannose or glucose in disrupted microsomes significantly stimulated the rate of formation of [¹⁴C]glucose, confirming previous findings (10, 15) that under these conditions the rates of the corresponding glucose-6-P-hexose phosphotransferase are significantly faster than the hydrolytic reaction. In contrast, the presence of mannose in the assay system for intact microsomes substantially reduced the rate of utilization of [¹⁴C]glucose-6-P.

The inhibition of glucose-6-P utilization by mannose can be rationalized in terms of the substrate transport hypothesis.
through an extension of the arguments presented earlier in an analysis of the kinetics of the glucose-6-P-glucose exchange activity of intact microsomes (1). The greater latency of the exchange activity compared with the glucose 6-P phosphohydrolase was viewed as the manifestation of product inhibition by unlabeled glucose-6-P, competing with substrate, [14C]glucose-6-P, within the micro-environment of the cisternal space. Since glucose-6-P can be translocated out of the intramicrosomal compartment, a limit is imposed on the accumulation of unlabeled glucose-6-P and therefore on the extent to which it can inhibit the utilization of labeled substrate. The synthesis of mannose-6-P within the cisternae would create a similar situation of product competing with substrate. However, in the absence of a route of efflux the level of cisternal mannose-6-P would exceed that of [14C]glucose-6-P, accounting for the greater inhibition.

Direct measurements were made of the glucose-6-P-mannose phosphotransferase activities of intact and disrupted microsomes (Table II). Data for the exchange activity and the mannose-6-P-glucose phosphotransferase, which confirm earlier findings (2, 10), are presented for comparison. The finding of a highly latent glucose-6-P-mannose phosphotransferase in intact microsomes is consistent with the conclusion that mannose-6-P generated in situ accumulates within the cisternae with the consequences that its utilization (i.e. hydrolysis) is favored, while its synthesis is restrained by product inhibition.

**Recommendation**—Since the initial discovery in this laboratory of the highly latent mannose-6-P phosphohydrolase (2, 26), we have used it as a routine means to evaluate the "integrity" of the glucose-6-phosphatase system in our microsomal preparations. While we suspected that the latency of this activity was related to the intactness of the microsomal permeability barrier (2), definitive evidence for this assertion awaited the demonstration in the present study of a clear quantitative correspondence between the activation of the low \( K_m \) mannose-6-P phosphohydrolase and the induction of EDTA permeability (Fig. 3). The potential value of using the latency of this activity as a general index of microsomal integrity warrants consideration. Assays of mannose-6-P hydrolysis before and after detergent supplementation can provide a quick and inexpensive means of assessing the quality of a given microsomal preparation and, for example, possible structural damage resulting from physical or chemical treatments of microsomal preparations as might occur during gradient centrifugation, gel chromatography, exposures to chemical probes to determine membrane sidedness, etc.

Regrettably, since neither EDTA permeability nor latency of mannose-6-P phosphohydrolase has been used in most investigations of glucose-6-phosphatase, the bulk of the existing literature on the enzyme cannot be applied with a high degree of confidence to judge the merits of the substrate transport hypothesis. Indeed, all future studies in which microsomal membrane permeability may be an important determinant should include an evaluation of the state of intactness of the microsomal preparations.

**Inherent Limitations in Evaluation of EDTA Permeability**—As shown in Fig. 4, the kinetic constants for the glucose-6-P phosphohydrolase activities of intact and disrupted microsomes are quite different. These differences are expressed at the time of loading microsomes with lead phosphate, reducing the reliability of EDTA permeability as a quantitative index of membrane intactness. The concentration of glucose-6-P (1 mM) initially present in the incubation medium used to generate cisternal lead phosphate precipitates is below \( K_m \) for intact microsomes, while it exceeds \( K_m \) for disrupted microsomes (see Fig. 4). Moreover, in the presence of 2 mM \( Pb^{2+} \), \( K_m \) of intact microsomes is increased, whereas lead ions do not affect the kinetics of glucose-6-P hydrolysis by disrupted preparations. Therefore, during incubation of microsomes with \( Pb^{2+} \) and glucose-6-P, a disproportionately high percentage of the precipitated lead phosphate is derived from substrate hydrolysis catalyzed by disrupted vesicles, which leads to overestimates of the fraction of vesicles permeable to EDTA. Thus, it is only in the extreme situations where the microsomes are either highly intact (see Fig. 3) or largely disrupted at the time of loading with lead phosphate that a quantitative correspondence exists between membrane intactness and the ability of EDTA to solubilize inorganic phosphate.

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**Table I**

**Effects of \( \alpha \)-glucose and \( \beta \)-mannose on rate of utilization of glucose-6-P by intact and disrupted microsomes**

<table>
<thead>
<tr>
<th>Hexose added</th>
<th>Glucose-6-P utilization</th>
<th>Intact microsomes</th>
<th>Disrupted microsomes</th>
<th>Latency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \mu )mol [14C]glucose formed/min/mg microsomal protein ( \times 10^{-4} )</td>
<td>( \times 10^{-4} )</td>
<td>( \times 10^{-4} )</td>
<td>( \times 10^{-4} )</td>
</tr>
<tr>
<td>None</td>
<td>0.11</td>
<td>0.23</td>
<td>26</td>
<td>1.1</td>
</tr>
<tr>
<td>0.2 M glucose</td>
<td>0.22</td>
<td>0.34</td>
<td>35</td>
<td>1.1</td>
</tr>
<tr>
<td>0.2 M mannose</td>
<td>0.13</td>
<td>0.40</td>
<td>67</td>
<td>1.1</td>
</tr>
</tbody>
</table>

*Activities are the mean values from duplicate assays for two experiments.

**Table II**

**Rat liver microsomal \( \alpha \)-glucose-6-P-exchange phosphotransferase activities**

The indicated phosphotransferase activities were assayed by determining the rates of incorporation of [14C]hexoses into the hexose-6-P products as described previously for the glucose-6-P-glucose phosphotransferase (exchange) activity (10). Assay mixtures (pH 6.5) contained the following in a final volume of 1.0 ml: 50 \( \mu \)mol of Tris-cacodylate buffer, 10 mg of bovine plasma albumin (defatted), 20 \( \mu \)mol of [14C]glucose-6-P (10^6 cpm/\( \mu \)mol), glucose or mannose as indicated, and 0.2 mg of microsomal protein before or after supplementation to 0.4% sodium taurocholate. [14C]Glucose was separated from the reaction mixtures and analyzed as described previously (10).
In our hands, the conditions described under “Experimental Procedure” for loading microsomes with lead phosphate are optimal. The kinetic limitations inherent in the system cannot be corrected by changing the concentrations of glucose-6-P or Pb**+. The fraction of lead phosphate deposited in EDTA-impermeable vesicles (i.e., the efficiency of phosphate trapping) appears to be determined by the following kinetic relationships: (a) diffusion of the lead ions into the cisternae; (b) P₄ formation within the cisternae; and (c) efflux of P₄ to the external medium. Maximal trapping efficiency occurs when P₄ formation is rate-limiting. A decrease in Pb**+ or an increase in glucose-6-P renders Pb**+ influx the slow step in the process, and a significant fraction of P₄ escapes to form lead phosphate precipitates outside the vesicles (i.e., in the EDTA-accessible space).

The enzyme of disrupted structures is more accessible to competitive inhibitors. This feature can be exploited to effect an increase in the relative proportion of lead phosphate deposited within intact vesicles. For example, the addition of 0.5 mM unlabeled mannose-6-P selectively inhibits the hydrolysis of glucose-6-P by disrupted microsomes (2) and consequently increases the proportion of ³²P, formed by intact vesicles. The inhibitor need not be a substrate for the intrinsic enzyme. Any compound which is a better inhibitor of the enzyme of disrupted microsomes can be used. Indeed, selective inhibition by maleate of glucose-6-P hydrolysis by disrupted microsomes may account for the comment of Gold and Widnell (7), who chose maleate over cacodylate for use in cytochemical studies because the latter buffer “changed the site of release of P, from the membrane in a small fraction of microsomal vesicles.” It should be obvious that the strategy of using selective inhibitors, while improving the results of cytochemical studies, must be avoided in assessments of the intactness of the membrane, since it would lead to underestimation of the degree of disruption.

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Microsomal membrane permeability and the hepatic glucose-6-phosphatase system. Interactions of the system with D-mannose 6-phosphate and D-mannose.
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