The Characteristics of Liver Glucose-6-phosphatase in the Envelope of Isolated Nuclei and Microsomes Are Identical*  

William J. Arion†, Leslie O. Schulz‡, Alex J. Lange, John N. Telford, and Heather E. Walls  

From the Division of Nutritional Sciences and the Section of Biochemistry, Molecular and Cell Biology of the Division of Biological Sciences, Cornell University, Ithaca, New York 14853  

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The abbreviations used are: ER, endoplasmic reticulum; TI, a glucose-6-P-specific translocase; T2, a phosphate translocase; T3, a relatively nonspecific phosphohydrolase with its active site at the luminal surface; and carbamyl-P, N-carbamyl-3'-diphosphoglycerate. The proportion of the two forms is easily quantified by assays of the "low mannose-6-phosphate activity" that is not expressed in untreated or intact microsomes. Latency is the percentage of activity in fully disrupted microsomes that is expressed only in disrupted structures (5, 7, 8). The enzymic characteristics of the coupled system which limit its capacity to catalyze net glucose-6-P synthesis (4, 6).

Nordlie and coworkers have forwarded a contrasting view in which the membrane influences the degree of latency of the associated phosphatase and phosphotransferase activity. Arion and coworkers (2-5) have demonstrated that glucose-6-P hydrolysis by intact microsomes involves the coupled functions of three integral components of the ER membrane: 1) a glucose-6-P-specific translocase, denoted T1, that mediates penetration of the hexose phosphate into the luminal cavity, 2) a relatively nonspecific phosphohydrolase with its active site at the luminal surface, and 3) a second translocase, denoted T2, that mediates P, efflux as well as slow rates of penetration of PP, and carbamyl-P through the membrane. Analyses from several laboratories indicate that glucose-6-P hydrolysis is the principal and probably the exclusive function of glucose-6-phosphatase of intact organelles in vivo (4, 10-12). The specificity of the enzyme in vivo with respect to its substrate and function appears to be restricted to glucose-6-P hydrolysis by a host of factors which include: (a) the selective transport properties of T1 and T2 (2, 4), (b) the physiologic concentrations of glucose-6-P, P, glucose, PP, and carbamyl-P (4), and (c) the intrinsic kinetic characteristics of the coupled system which limit its capacity to catalyze net glucose-6-P synthesis (4, 6).

Our results show clearly that apparent differences in the glucose-6-phosphatase activity of untreated preparations of nuclei and microsomes are simply expressions of significant differences in the degree of intactness of their respective permeability barriers. Since flattened cisternae, characteristic of the rough endoplasmic reticulum in situ, are preserved in intact regions of the envelope of isolated nuclei, the present findings constitute the most direct and definitive evidence to date that the properties of glucose-6-phosphatase in the endoplasmic reticulum in situ are faithfully reproduced with intact microsomes.

Conflicting perceptions prevail as to the role of the ER1

1 The abbreviations used are: ER, endoplasmic reticulum; T1, a glucose-6-P-specific translocase that mediates the penetration of the hexose phosphate into the ER cisternae; T2, a phosphate translocase that mediates efflux of P; NEM, N-ethylmaleimide; protease, the nonspecific protease from Bacillus amyloliquefaciens (i.e. "nagarse"); Buffer A, 0.25 M sucrose/5 mM Tris acetate, pH 7.4; Buffer B, 0.25 M sucrose/5 mM Tris acetate, pH 7.4; Buffer C, 0.25 M sucrose/5 mM Tris chloride/25 mM KCl/5 mM MgCl2, pH 7.4; Buffer D, 0.25 M sucrose/50 mM Tris chloride/25 mM KCl/5 mM MgCl2, pH 7.4.

2 Latency is the percentage of activity in fully disrupted microsomes that is expressed only in untreated or intact microsomes. Latency is calculated as 100 (activity in disrupted membranes - activity in intact or untreated membranes)/activity in disrupted membranes.

3 In this paper, microsomes or nuclei isolated from liver homogenates, washed and assayed without further treatment are referred to as "untreated." As noted previously (4-6, 8), untreated microsomes are heterogeneous preparations composed of intact vesicles ("intact microsomes") in which the limiting membrane acts as a selective permeability barrier and disrupted structures in which selective permeability is lacking and the enzyme has free access to ionic substrates and inhibitors. The proportion of the two forms is easily quantified by assays of the "low K," mannose-6-phosphatase activity that is expressed only in disrupted structures (5, 7, 8). The enzymic activity of intact microsomes is calculated as the activity of untreated microsomes minus the contribution of enzyme in the disrupted component (4-6).

4 Untreated microsomes are converted to fully disrupted microsomes by treatments with detergents or NH4OH which completely destroy the selective permeability of the membrane (2, 3, 8, 9). The definitions of "untreated" and "intact" nuclear membranes are given in the text.

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of the physiologic function of glucose-6-phosphatase which advocates an important role for the associated phosphotransferase activity (see Refs. 13–16 for reviews). These investigators have proposed that the relatively high latency of the phosphotransferase activity observed with untreated homogenates and microsomes arises through imposition of artificial constraints on the enzyme or translocases as a consequence of the morphological changes that occur when microsomes are generated from fragments of the ER. In support of this thesis they have reported that the phosphohydrolase and phosphotransferase activities are significantly more latent in microsomes than in isolated hepatic nuclei (17–19) or in rat hepatocytes rendered permeable to ionic substrates by exposure to filipin (20, 21). However, the relevance of their observations rests on the validity of the underlying assumption that the native structure of the ER and, therefore, the function of glucose-6-phosphatase in situ are retained in the envelope of isolated nuclei and in filipin-treated hepatocytes.

The “integrity” or degree of “intactness” of the selective permeability barrier defined by the single limiting membrane of hepatic microsomes can be quantified either by measuring EDTA permeability (8, 22) or more confidently by determining the latency of the “low K+” mannose-6-P phosphohydrolase activity catalyzed by glucose-6-phosphatase (5, 7, 8). These criteria are now used routinely in studies with hepatic and renal microsomes. By contrast, except for the apparent preservation of gross morphology seen in electron micrographs of isolated nuclei (18), the intactness of the permeability barrier defined by the nuclear envelope has not been evaluated. Likewise, no information is available as to whether or not the structure or permeability characteristics of the ER in hepatocytes exposed to filipin. Thus, it remained to be established whether studies with either of these experimental models of the “ER in situ” generate reliable data on the characteristics of glucose-6-phosphatase in the intact cell. The characteristics of the nuclear glucose-6-phosphatase system are presented here. Results of a parallel study of filipin-treated rat hepatocytes will be presented elsewhere.

EXPERIMENTAL PROCEDURES

RESULTS AND DISCUSSION

Quantitative Assessment of the "Intactness" of the Envelope of Isolated Nuclei—Mannose-6-P phosphohydrolase activity routinely was between 40 and 60% latent in our nuclear preparations (Table I and below). In our experience maximal expression of nuclear mannose-6-phosphatase activity and other activities catalyzed by glucose-6-phosphatase required exposure of nuclear preparations to 0.1% sodium deoxycholate rather than the 0.05% concentration routinely used by others in their characterizations of the enzyme in nuclear membranes (17–19). The activity of mannose-6-phosphatase in nuclei supplemented with 0.05% deoxycholate averaged only 72.0 ± 5.8% (n = 32; range = 58.9 to 82.7%) of the value determined with preparations supplemented to 0.1% deoxy-

| Table 1 |

| Correlation between the latency of mannose-6-phosphatase and EDTA impermeability in the envelope of isolated rat liver nuclei |

Mannose-6-phosphatase was assayed at pH 6.5 with 1 mM substrate. EDTA permeability was quantified by measuring its ability to solubilize P, from lead phosphate precipitates formed during prior incubation of nuclei with 1 mM glucose-6-P and 2 mM Pb(NO₃)₂ (see under “Experimental Procedures” in the miniprint).

| Latency (%) |
|------------|
| 1. Mannose-6-phosphatase | nmol P/min/mg protein |
| Untreated nuclei (A) | 12.6 ± 4.2 |
| Deoxycholate-treated nuclei (B) | 29.9 ± 8.1 |
| Latency = (1 - A/B) x 100 | 58.1 ± 5.2 |
| 2. Impermeability to EDTA | % Inaccessible lead phosphate |
| Untreated nuclei | 59.8 ± 12.7 |

* Values are the means ± S.E. from five separate experiments.

b Nuclear suspensions were supplemented to 0.1% sodium deoxycholate to obtain full expression of mannose-6-phosphatase activity.

Table 1

A Model of the Envelope of Isolated Nuclei—The characteristics of mannose-6-phosphatase and EDTA permeability strongly suggest that the envelope of isolated nuclei does not define a continuous permeability barrier. We believe that the preceding results and those that follow are most readily explained in terms of the schematic drawn in Fig. 3. Ultrastructural studies of intact cells show clearly that the membranes of the ER and nuclear envelope constitute a morphologic continuum in situ and that the continuum is disrupted when tissues are homogenized (23–28). Whether the envelope in an isolated nucleus defines an intact or a disrupted permeability barrier is likely determined by the location of the sites at which disruption occurs between the ER proper and the outer aspect of the envelope. If the location is in a region of the ER proper (e.g., at points designated by arrows labeled A in the upper panel of Fig. 3), the broken membranes associated with the envelope would have the freedom to contact one another and fuse. Disruption and fusion in this case would generate a region of the envelope bounded by a continuous permeability barrier, as illustrated by the intact area in the lower panel of Fig. 3, and it would account for the “blebs” that are occasionally seen in the envelope of isolated nuclei (23, 27). Alternatively, if disruption occurs at loci on the envelope proper (e.g., at arrows labeled B in the upper panel of Fig. 3), it is expected that the physical restriction imposed by association of the inner aspect of the envelope (the so-called inner membrane) with the chromatin network (23, 24, 26) would prevent association and, therefore, fusion of the broken membranes. This
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FIG. 1. Electron micrograph showing the localization of cytochemical reaction product in the perinuclear cisternae after washing nuclei with 4 mM EDTA in buffer D (0.25 M sucrose/50 mM Tris-HCl/25 mM KCl/4 mM MgCl₂, pH 7.4). Magnification, bar equals 1 μ. Other details are given under "Experimental Procedures" in miniprint.

would generate a region in the envelope where the permeability barrier is interrupted (designated leaky in the lower panel of Fig. 3). The components localized within this region, which is the perinuclear cisterna in situ, would have free but artificial access to ionic metabolites (e.g. mannose-6-P and EDTA) and macromolecules (see below). Such interrupted regions (i.e. gaps) in the envelope have frequently been noted in electron micrographs of isolated nuclei (23, 25-28).

The scheme in Fig. 3 formed the basis for our experimental approach to the characterization of glucose-6-phosphatase in isolated nuclei. Implicit in the model is the existence of two "glucose-6-phosphatase activities" in untreated nuclei, one activity representing the coupled system (the intact component) and the other the enzyme in disrupted membranes. Accordingly, the kinetics of glucose-6-P hydrolysis in microsomes and nuclei was compared in fully disrupted and intact membranes. The latter data were obtained by subtracting the contribution of the "disrupted component" from the activities determined for untreated membrane preparations as detailed under "Experimental Procedures" in the Miniprint.

Competitive Interactions of Nuclear Glucose-6-phosphatase with Mannose-6-P and Glucose-6-P—The experiment summarized in Table II was carried out to provide an additional test of the proposition that latency of mannose-6-P hydrolysis provides a reliable quantitative measure of the degree of intactness of the nuclear envelope. The competitive interactions between mannose-6-P and glucose-6-P were studied before and after supplementing nuclei to 0.1% deoxycholate, i.e. in untreated and fully disrupted membranes. The data for fully disrupted membranes define the situation in which both substrates have complete and equal access to the active sites of the phosphohydrolase. Whereas glucose-6-P was equally effective in inhibiting mannose-6-P hydrolysis in untreated and disrupted preparations, mannose-6-P was substantially less effective in inhibiting glucose-6-P hydrolysis by untreated compared with fully disrupted nuclei. If it is assumed that mannose-6-P interacted only with the enzyme in disrupted membranes (i.e. 48% of the total enzyme), the observed inhibition of glucose-6-P hydrolysis catalyzed by the disrupted component in untreated nuclei (45%) agrees well with the expected value (39%) calculated from the 82% inhibition of glucose-6-phosphatase in fully disrupted nuclei.

The observations with rat liver nuclei reproduce earlier
findings with microsomes from rat liver (7) and human liver (5). They strongly support the validity of using latency of mannose-6-P hydrolysis to quantify the intactness of the nuclear envelope in hepatocytes.

Influences of Protease Treatment on Glucose-6-phosphate Activity in Microsomes and Nuclei—Dallner and coworkers (3, 29, 30) have used proteolytic enzymes to probe and define the transverse topology of the ER membrane. The finding that glucose-6-phosphatase can be inactivated only when the protease can penetrate into the luminal compartment (e.g., after treatment with deoxycholate) demonstrates that the active site of the enzyme is situated at the luminal surface. Measurements of EDTA permeability and latency of mannose-6-P hydrolysis (Table I) suggested that approximately 50% of the glucose-6-phosphatase in the nuclear envelope is localized in "leaky" membranes and accordingly should be accessible to added proteases. Therefore, the influences of protease on glucose-6-phosphatase of isolated nuclei were studied with two objectives in mind: 1) to verify the susceptibility of enzyme in leaky membranes to proteolytic degradation and 2) to compare the responses of the glucose-6-phosphatase system in intact microsomes and the intact nuclear envelope to proteolytic treatments.

The procedure for treatment with protease was first evaluated in studies with intact microsomes and microsomes rendered freely permeable to macromolecules by prior exposure to 0.1 M NH₄OH (3) (see Table III and accompanying text). The effects of protease treatment on nuclear glucose-6-phosphatase are summarized in Table IV. The increased latency of mannose-6-P hydrolysis following exposure to the protease was expected. It is predictable from Fig. 3 that protease treatment would preferentially destroy the enzyme in leaky membranes and thus increase the proportion of enzyme localized within intact regions of the recovered membranes. The effects of protease treatment on the kinetics of glucose-6-P hydrolysis were qualitatively and quantitatively similar to those observed with microsomes (Table III); protease treatment increased the apparent $K_m$ for glucose-6-P in intact but not disrupted membranes. This was accompanied by increased latency of glucose-6-phosphatase activity, especially in media containing 2 mM glucose-6-P.

Comparison of the Effect of N-Ethylmaleimide on Microsomal and Nuclear Glucose-6-phosphatase Activity—Exposure of microsomes to NEM inhibits glucose-6-P hydrolysis by intact microsomes (31, 32). The inhibition is abolished when NEM-treated microsomes are subsequently disrupted (e.g. with detergents). At neutral pH, 1 mM or less of the sulphydryl poison causes no inhibition when incubated with the enzyme of fully disrupted microsomes (31, 32). Inhibition by NEM is expressed as an increase in the Michaelis constant for glucose-6-P without a change in $V_{max}$ (31). Moreover, the inhibition can be specifically prevented by the presence of glucose-6-P at concentrations greater than 50 mM; mannose-6-P, Pi, and D-glucose have no protective influence at concentrations as high as 0.2 M. These observations indicate that NEM reacts with a thiol group located at or near the glucose-6-P-binding site on the translocase.

Table V compares the influence of NEM on glucose-6-phosphatase of nuclei and microsomes. The thiol poison had no effect on glucose-6-phosphatase activity in preparations that were disrupted after exposure to NEM, and the latency of mannose-6-phosphatase was unaltered by NEM exposure. When activity values obtained in the absence of deoxycholate supplementation were corrected for the contribution of disrupted component, the latency of glucose-6-P hydrolysis was identical in intact microsomes and intact nuclear membranes before NEM treatment, and the thiol poison caused identical inhibitions in nuclei and microsomes.

Carbamyl-P-Glucose Phosphotransferase Activity—Gunderson and Nordlie (17, 18) have concluded that all phosphohydrolase and phosphotransferase activities catalyzed by glucose-6-phosphatase are much less "constrained" (i.e. less latent) in nuclei than in microsomes. The data in Table VI compare the activity levels of carbamyl-P-glucose phosphotransferase in nuclei and microsomes before and after disruption with optimal concentrations of sodium deoxycholate. The results confirm that carbamyl-P-glucose phosphotransferase, like mannose-6-phosphatase, appears much less latent in untreated nuclei as compared with untreated microsomes. However, when the phosphotransferase activities in the untreated membranes are adjusted for the contribution of enzyme in leaky membranes, virtually identical and extremely high latencies are observed for the two preparations.

Table IV

| Treatment condition | No protease | Plus protease |
|---------------------|-------------|--------------|
| Latency of mannose-6-P | Hydrolysis, % | | |
| 60.1 | 82.8 |
| Milliliters of recovered activity | | |
| 20 mM glucose-6-P | | |
| Intact membranes | 289 | 100 |
| After deoxycholate supplementation (Latency, %) | 393 | 252 |
| 2 mM glucose-6-P | | |
| Intact membranes | 153 | 27.3 |
| After deoxycholate supplementation (Latency, %) | 320 | 198 |
| Estimates of $K_m$, mM | | |
| Intact membranes | 2.2 | 8.4 |
| After deoxycholate supplementation | 0.53 | 0.63 |

a Because of the obvious influence of protease treatment on milligrams of nuclear protein, activities are expressed as "recovered milliliters" rather than units/mg of protein (see Table III).

b Estimates of $K_m$ are in part based on a limited number of experiments.

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TABLE V

Latency and sensitivity to N-ethylmaleimide of glucose-6-phosphatase in nuclear and microsomal membranes before and after correcting for the disrupted component.

The means of three determinations in each of two experiments are presented. See under "Experimental Procedures" in "miniprint" for details of NEM treatment. Assay conditions were as described in Table II, except 1 mM glucose-6-P was used.

| Parameters (Latency/activity/% inhibition) | Nuclei | Microsomes |
|------------------------------------------|--------|------------|
|                                          | Control | NEM-treated | Control | NEM-treated |
| A. Latency of mannose-6-phosphatase (%) |         |             |         |             |
| Total glucose-6-phosphatase activity (nmol/min/mg protein) | 46.1 ± 3.6 | 47.1 ± 2.5 | 94.9 ± 0.1 | 94.4 ± 0.3 |
| =Deoxycholate                          | 34.6 ± 2.2 | 25.0 ± 1.3 | 160 ± 12 | 27.6 ± 1.4 |
| +Deoxycholate                          | 49.0 ± 1.6 | 46.8 ± 4.7 | 421 ± 9 | 414 ± 1 |
| Apparent inhibition by NEM (%)         |         |             |         |             |
| =Deoxycholate                          | 27.7 ± 4.2 | 83.7 ± 1.2 |
| +Deoxycholate                          | 3.7 ± 2.0 | 1.7 ± 2.2 |
| C. "Intact" glucose-6-phosphataseb (nmol/min/mg protein) | 17.8 ± 2.1 | 5.02 ± 0.14 | 155 ± 15 | 4.7 ± 1.5 |
| Inhibition by NEM (%)                  |         |             |         |             |
| Latency of intact system (%)           | 63.4 ± 9.2 | 62.6 ± 1.3 |

a Indicates before (=deoxycholate) and after (+deoxycholate) supplementation of nuclei with 0.1% or microsomes with 0.2% sodium deoxycholate.

b The "intact component" was normalized to 100% of the enzymic activity of deoxycholate-treated preparations.

TABLE VI

Comparison of carbamyl-P:glucose phosphotransferase activities in hepatic nuclei and microsomes from a 24-h fasted rat.

Assay media, pH 7.0, contained in a volume of 1.0 ml: 50 mM Tris/cacodylate buffer, 10 mg of bovine plasma albumin, 100 mM glucose, and 5 mM carbamyl-P (phosphotransferase assays only) or 1 mM mannose-6-P (phosphotransferase assays), and microsomes before and after supplementation to 0.2% deoxycholate (144 and 36 µg of protein, respectively) or nuclei before and after supplementation to 0.1% deoxycholate (205 and 103 µg of protein, respectively). Microsomes and nuclei were isolated from the same liver homogenate. The means of 3 determinations are presented.

| Membrane preparation | Untreated | Disrupted | Intact* | Untreated | Intact* |
|----------------------|-----------|-----------|---------|-----------|---------|
|                      | millilunits/mg protein % |             |         |             |         |
| A. Carbamyl-P:glucose phosphotransferase activity |         |             |         |             |         |
| 1. Microsomes        | 75.0      | 803        | 33.2    | 90.7      | 95.9    |
| 2. Nuclei            | 30.6      | 71.1       | 1.23    | 57.0      | 98.3    |
| B. Mannose-6-P phosphohydrolase activity |         |             |         |             |         |
| 1. Microsomes        | 21.8      | 402        | 94.7    | 58.0      |         |
| 2. Nuclei            | 18.4      | 45.8       |         |           |         |

* The intact component was calculated and normalized to 100% of the enzymic activities of deoxycholate-supplemented preparations (see under "Experimental Procedures").

Optimal concentration of detergent (i.e. 0.2%) are given. Results are presented for phosphohydrolase activities observed before correcting for the contribution of the disrupted component (Table VIII) and after such corrections were made (Table IX).

Mannose-6-P hydrolysis was at least 94% latent in whole homogenates, and its latency was not significantly altered by the changes in endocrine or nutritional status of the rats (Table VIII, part B). In confirmation of earlier findings (reviewed in Refs. 13 and 33), the latency of glucose-6-phosphatase activity was markedly influenced by changes in endocrine and nutritional status. Latency of glucose-6-phosphatase in liver homogenates was increased by fasting and especially diabetes, whereas cortisol injection caused only a modest and statistically insignificant increase in latency activity. In all groups, the phosphohydrolase activities in isolated nuclei were only about one-half as latent as observed for the homogenates (Table VIII, part B). However, when activity values for glucose-6-P hydrolysis by untreated preparations were corrected for the disrupted component, essentially identical responses to treatment are seen in intact membranes of nuclei and homogenates (Table IX).

The percentage of nuclei recovered from whole homogenates was determined by analyses of DNA based on the assumption that 98.5% of the liver DNA is localized in the nucleus (34). Mean recoveries ranged from 39% for 24-h fasted rats to 67% for diabetic animals (Table VII). These recoveries are similar to those reported by others (26, 27, 35). When the percentage of recovery of nuclear DNA was used to normalize the nuclear phosphohydrolase activities to 100% recovery, the data (Table IX, part C) show that only about 2% of the total hepatic glucose-6-phosphatase is localized in the nuclear envelope. The much higher value reported previously (18, 19) appears to have originated from a calculation using an estimate (from Ref. 36) of the protein content of a crude nuclear fraction (i.e. a 600 x g pellet) rather than purified nuclei. Our findings support the conclusion of Kartenbeck et al. (26) that the nuclear envelope can be quantitatively neglected in consideration of the overall rate of glucose formation from glucose-6-P in the rat hepatocyte.

The smallest percentage of total liver activity was seen in nuclei from the streptozotocin-diabetic rats. Garfield and Cardell (37) have reported that experimental diabetes increases the proportion of total hepatic glucose-6-phosphatase activity in the smooth ER. Therefore, the lower fraction of liver glucose-6-phosphatase in the nuclei from diabetic rats is expected, since the nuclear envelope is an extension of the rough ER (23–26).
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**TABLE VIII**

*Phosphohydrolase activities in liver homogenates and isolated nuclei before correction for the contribution of enzyme in "leaky" membranes*

Assay media contained the following in a volume of 1 ml: 50 mM Tris/cacodylate buffer, pH = 6.5, 10 mg of bovine plasma albumin (defatted), 10 mM phosphate substrates, and appropriate amounts of homogenate or nuclear suspension to measure initial rates. Other details are described under "Experimental Procedures."

| Preparation/activity | Treatment group |
|----------------------|-----------------|
|                      | Normal fed (n = 4)* | 24-h fasted (n = 4) | Fed diabetics (n = 3) | Cortisol-treated (n = 4) |
| A. Enzymic activities** | units/g liver | | | |
| Liver homogenates: Glucose-6-phosphatase | | | | |
| - Deoxycholate | 8.82 ± 0.36 | 15.3 ± 0.9 | 17.4 ± 1.9 | 15.8 ± 1.2 |
| + Deoxycholate | 12.6 ± 0.8 | 29.1 ± 1.2 | 49.1 ± 9.6 | 25.1 ± 4.4 |
| Mannose-6-phosphatase | | | | |
| - Deoxycholate | 0.67 ± 0.09 | 1.48 ± 0.39 | 1.10 ± 0.15 | 0.88 ± 0.16 |
| + Deoxycholate | 11.2 ± 2.0 | 27.5 ± 1.2 | 44.7 ± 9.5 | 23.3 ± 4.4 |
| Isolated nuclei: Glucose-6-phosphatase | | | | |
| - Deoxycholate | 0.223 ± 0.058 | 0.433 ± 0.052 | 0.433 ± 0.123 | 0.441 ± 0.118 |
| + Deoxycholate | 0.268 ± 0.051 | 0.576 ± 0.039 | 0.613 ± 0.164 | 0.563 ± 0.130 |
| Mannose-6-phosphatase | | | | |
| - Deoxycholate | 0.126 ± 0.017 | 0.269 ± 0.031 | 0.277 ± 0.055 | 0.197 ± 0.067 |
| + Deoxycholate | 0.242 ± 0.041 | 0.534 ± 0.062 | 0.569 ± 0.161 | 0.509 ± 0.179 |
| B. Apparent latency | | | | |
| Glucose-6-phosphatase | | | | |
| Homogenates | 30.0 ± 2.1 | 47.8 ± 1.4 | 64.6 ± 3.9 | 37.1 ± 6.4 |
| Isolated nuclei | 15.5 ± 2.4 | 24.8 ± 4.7 | 27.7 ± 6.8 | 21.7 ± 0.6 |
| Mannose-6-phosphatase | | | | |
| Homogenates | 94.0 ± 0.6 | 94.6 ± 1.2 | 97.5 ± 0.8 | 96.2 ± 0.6 |
| Isolated nuclei | 47.9 ± 4.1 | 49.6 ± 2.4 | 51.3 ± 4.1 | 61.3 ± 5.4 |

* n = number of animals in each treatment group.
** Activity determined before (+deoxycholate) and after (-deoxycholate) supplementation of homogenates or nuclear suspensions to 0.2% and 0.1%, respectively, with sodium deoxycholate.
*** Normalized to 100% recovery based on DNA in homogenate and nuclei (see Table VII).

**TABLE IX**

*Comparison of total glucose-6-phosphatase activity in liver homogenates and isolated nuclei after correction for the contribution of enzyme in "leaky" membranes*

Enzymic activities in Table VIII were corrected for the contribution of disrupted component as detailed under "Experimental Procedures." Activities in intact membranes were normalized to correspond to percentage recovery using values for percent of recovery of liver DNA in isolated nuclei (see Table VII).

| Preparation/activity | Treatment group |
|----------------------|-----------------|
|                      | Normal fed (n = 4)* | 24-h fasted (n = 4) | Fed diabetics (n = 3) | Cortisol-treated (n = 4) |
| A. Glucose-6-phosphatase activity | units/g liver | | | |
| Liver homogenates: Intact membranes | 8.61 ± 0.31 | 14.6 ± 0.6 | 16.6 ± 2.1 | 15.5 ± 1.1 |
| Fully disrupted | 12.6 ± 0.8 | 29.1 ± 1.2 | 49.1 ± 9.6 | 25.1 ± 4.4 |
| Isolated nuclei: Intact membranes | 0.171 ± 0.041 | 0.288 ± 0.068 | 0.289 ± 0.119 | 0.355 ± 0.154 |
| Fully disrupted | 0.268 ± 0.051 | 0.576 ± 0.039 | 0.613 ± 0.164 | 0.563 ± 0.130 |
| B. Latency | % | | | |
| Liver homogenates | 31.8 ± 4.8 | 50.4 ± 1.7 | 65.8 ± 3.4 | 37.6 ± 6.6 |
| Isolated nuclei | 36.3 ± 11.0 | 50.3 ± 9.0 | 54.3 ± 12.6 | 36.5 ± 15.2 |
| C. Nuclear activity as per cent of whole liver activity | | | | |
| Intact | 2.0 ± 0.4 | 2.0 ± 0.5 | 1.7 ± 0.7 | 2.3 ± 0.9 |
| Fully disrupted | 2.1 ± 0.4 | 2.0 ± 0.2 | 1.2 ± 0.2 | 2.2 ± 0.4 |

* n = number of rats in each group.

CONCLUSIONS

The most significant observations of the present study are presented below.

First, whereas in a gross morphologic sense isolated nuclei may be considered "intact" (18), measurements of mannose-6-phosphatase latency, EDTA permeability, and the susceptibility of the enzyme to protease-catalyzed inactivation all indicate that the envelope of isolated nuclei is significantly disrupted. This conclusion is supported by the results of extensive ultrastructural examinations of isolated nuclei which clearly show interruptions (i.e. gaps) in the outer nuclear membrane (18, 25–28).

Second, regions of the envelope where a permeability barrier to EDTA does exist were confirmed by electron microscopy (Fig. 1). Thus there are regions of the envelope where the membrane forms a continuous permeability barrier which blocks free access of solutes in the surrounding medium to the perinuclear cisternae.

Third, in all comparisons, the characteristics of the glucose-
6-phosphatase activities in both intact and fully disrupted nuclear membranes were virtually identical with their microsomal counterparts. The comparisons include the Michaelis constant for glucose-6-P, the quantitative and qualitative effects of exposures to mannose-6-P, NEM, and protease, and the latency of carbamyl-P:glucose phosphotransferase, and the patterns of response of activity and change in latency of glucose-6-phosphatase induced by fasting, diabetes, and cortisol injection.

These findings bear directly and decisively on the questions that have been raised concerning the relevance of the characteristics of glucose-6-phosphatase in intact microsomal vesicles to an understanding of the form and the function of the enzyme as it occurs in vivo (13-16). The concept that glucose-6-phosphatase is artificially constrained in intact microsomal vesicles originated (13) as an attempt to explain the phenomenon of latency which is characteristic of the various phosphohydrolase and phosphotransferase activities catalyzed by the enzyme. With the exception of activities with glucose-6-P and the PP, phosphohydrolase, all activities catalyzed by glucose-6-phosphatase of fully disrupted preparations are either highly or completely suppressed in intact microsomes (4, 7, 8). A position similar to Nordlie and his coworkers has been taken by Wishart and Fry (28), who have suggested that the latency of UDP-glucuronosyltransferase (EC 2.4.1.17) observed in microsomes, but not isolated nuclei, is largely a preparative artifact resulting from vesicularization of the ER membrane.

The argument that latency of these enzymes in microsomes results from "morphological constraints" imposed by the membrane fails to consider the role of the ER membrane as a permeability barrier (8, 22, 30, 38-40). Selective permeability is a basic tenet of the substrate transport model of glucose-6-phosphatase, which explains latency in terms of the specificity of the transporters, T_1 and T_2, and the kinetics of the transport-dependent system (2, 4-6, 8, 38-40). The data reported here clearly that differences observed between untreated preparations of nuclei and microsomes are simply expressions of significant differences in the degree of intactness of their respective permeability barriers.

Since flattened cisternae, characteristic of the ER in situ, are present in the intact regions of isolate nuclei (23-27), a comparison of nuclei and microsomes provides a direct unambiguous test of whether fragmentation and vesicularization of the ER membrane artificially alters glucose-6-phosphatase. Thus, the demonstration that the characteristics of the glucose-6-phosphatase system in intact membranes of these preparations, in fact, are identical constitutes the most persuasive evidence to date that the properties of glucose-6-phosphatase in situ are faithfully reproduced with intact microsomes. We find no merit in the arguments (13-16) that the enzyme or translocases of the glucose-6-phosphatase are preserved in the intact regions of the envelope of isolated nuclei (23-27), a comparison of nuclei and microsomes prepared by centrifugation of the ER membrane.

It remains our view (Refs. 4 and 7 and see the "Introduction") that glucose-6-P hydrolysis is the only significant physiologic function of glucose-6-phosphatase.

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Glucose-6-phosphatase in the Nuclear Envelope

W. J. Aronson, L. O. Schulz, A. J. Langer, J. W. Pfeiffer, and H. E. Walls

Enzymological Procedures

Assay and Preparation of Nuclear Envelopes - Male, long-tailed rats between 170 and 220 g were obtained from Blue Spruce Farms (Stillwater, N.J.) and used in all studies. Reagents for inducing experimental disturbances with streptomycin (44) and cortisol treatment (45) were as described earlier. In all cases liver homogenates were prepared as 10 % (w/v) in a Potter-Elvehjem homogenizer fitted with a motor-driven nylon pestle operating at 600 rpm. The duration of homogenization was 30 seconds, equivalent to 3 or 4 strokes per min. Homogenates were prepared from 10% liver homogenates essentially as described by Aronson and Aronson (48), except the medium for homogenization was buffer A (i.e., 0.25 M sucrose, 5 mM Tris-HCl, pH 7.4). Microscopic and morphological examination of buffer A (i.e., 0.25 M sucrose, 5 mM Tris-HCl, pH 7.4) was performed after homogenization in buffer B (i.e., 0.25 M sucrose, 50 mM Tris-acetate, 75 mM KCl, 5 mM MgCl₂) and centrifugation at 100,000 rpm for 60 min. Nuclei isolated from homogenates were identified by the nuclei (see Materials and Methods). Nuclei isolated from homogenates were identified by the nuclei (see Materials and Methods). Nuclei isolated from homogenates were identified by the nuclei (see Materials and Methods).

Activity of Glucose-6-phosphatase in the Nuclear Envelope

The activity of glucose-6-phosphatase in the nuclear envelope was determined by measuring the level of hexokinase activity. The enzyme was assayed in the presence of 10 mM glucose-6-phosphate and 5 mM MgCl₂. After 20 min at 37°C, the reaction was stopped by the addition of 0.1 N HCl to a final concentration of 5 N HCl. The reaction was performed at 37°C in the presence of 10 mM glucose-6-phosphate and 5 mM MgCl₂. After 20 min at 37°C, the reaction was stopped by the addition of 0.1 N HCl to a final concentration of 5 N HCl. The reaction was performed at 37°C in the presence of 10 mM glucose-6-phosphate and 5 mM MgCl₂. After 20 min at 37°C, the reaction was stopped by the addition of 0.1 N HCl to a final concentration of 5 N HCl. The reaction was performed at 37°C in the presence of 10 mM glucose-6-phosphate and 5 mM MgCl₂. After 20 min at 37°C, the reaction was stopped by the addition of 0.1 N HCl to a final concentration of 5 N HCl. 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Glucose-6-phosphatase in the Nuclear Envelope

The results, summarized in Table III, are in full agreement with the earlier findings of慧龙等. More than 90% of the enzyme associated with membranes lacking a selective permeability barrier was inactivated by protein treatment. 90% of the enzyme was lost in intact of detergent-disrupted microsomes, 75% of the enzyme survived exposure of untreated microsomes to protease (Table III, part A). The unchallenged intact of detergent-disrupted microsomes (Table III, part A) implies that permeability of membranes was unimpaired by exposure of untreated microsomes to protease. The data show, however, that protease treatment preferentially inactivated glucose-6-phosphatase of intact membranes, since 50% and 98% of enzyme were observed in assays at 1 and 30 min, respectively.

# Results

### Validation of measurements of HEP permeability

Two aspects of the cytophysical experiments required further study. First, the well-documented requirement for MgCl2 to preserve nuclear structure and organization (31, 32) was confirmed in the present study (data not shown). Consequently, the cytophysical experiments were carried out in intact Percoll-purified 4 mg/mL microsomes. In this manner, valid assessment of HEP permeability could still be obtained (see below), because of the markedly greater binding affinity of MgCl2 for HEP compared with MgCl2 (33). Second, in an earlier examination of HEP permeability in sub-4-m2 membrane was raised that HEP permeability may not be a reliable qualitative index of membrane integrity when the preparations under study are appreciably disrupted (i.e., lacking selective permeability) before isolation with glucose-6-phosphatase and lead time. Specifically, the possibility was addressed that disruption of microsomes with MgCl2 and glucose-6-phosphatase activity of disrupted membranes (as an increase kinetic advantage over the activity in intact membranes. If this were true, it would favor disruption of cytoplasmic product in disrupted membranes and thus lead to overestimation of the degree of disruption (see Ref. 8 for detail). Since a relatively high degree of membrane disruption in nuclei was suggested by the latency of glucose-6-phosphatase (Table I), the correlation between HEP permeability and mannose-6-phosphate was subjected to further study.

### Subcellular Microenvelope

The experiment presented in Fig. 1 was designed to test (a) whether 4 mg/mL MgCl2 interfered with the assessment of HEP permeability and (b) whether the degree of enzyme disruption of the membranes would influence the correlation between latency of glucose-6-phosphatase and the impermeability of the membranes to HEP. Intact microsomes (1 mg/mL) and microsomes disrupted with 0.2 M KCl (31, 32, 41) were used to generate membrane preparations with different proportions of intact and disrupted components. Two attributes of the MgCl2-disrupted microsomes make them a suitable preparation for this study. First, they lack a permeability barrier to mannose-6-phosphate as evidenced by the absence of latency with this substrate (refs. 3 and 21). Second, lead phosphate deposition in cytophysical studies is completely reversible to HEP (see Fig. 2). Incubation with glucose-6-phosphatase and MgCl2 was carried out in buffer B (0.25 M sucrose, 5 mM HEPES, pH 7.4) and in buffer used in cytophysical studies (buffer, 1 mg/mL HEP was assayed as described in the legend to Fig. 3). A linear, linear correlation between the impermeability of the preparations to HEP (i.e., HEP-inaccessible lead phosphate) and the fraction of intact mammalian microsomes was observed for both buffer systems. This correlation was especially good for preparations with intact fractions of mammalian microsomes greater than 40%, which covers the range observed for all preparations of nuclei used in the present study.

### Table III

The effect of treatment with protease on glucose-6-phosphatase activity

| Treatment Condition | No protease | Protase
|---------------------|-------------|---------|
| A. Intact microsomes |             |         |
| 1. Latency of mannose-6-phosphate, s | 92.4 | 93.4 |
| 2. Units of recovered activity* | 2.0 | 370 |
| Intact microsomes | 1.25 | 1.13 |
| After demembranation supplementation | 1.45 | 1.35 |
| (Latency, s) | 160.25 | 155.25 |
| B. 3 mg/mL glucose-6-phosphate | 1.06 | 0.44 |
| Intact microsomes | 1.05 | 1.05 |
| After demembranation supplementation | 1.00 | 1.05 |
| (Latency, s) | 10.45 | 10.75 |
| C. Emission of 45Ca, % Intact microsomes | 2.0 | 2.0 |
| After demembranation supplementation | 0.57 | 0.57 |

### Figure 2

Correlation between the latency of mannose-6-phosphate and EG6 phosphatase in intact and disrupted microsomes. A, microsomes in buffer B, 0 mM sucrose in buffer B (see text). R2 is the correlation coefficient. Mixtures corresponding to 5, 20, 40, 60, 80, and 100% intact microsomes were made in buffer B or buffer B by combining untreated microsomes (4.4 mg protein/m) and MgCl2-disrupted microsomes (12.8 mg protein/m) in appropriate proportions. Each mixture (115 ml) was incubated with 2 ml of buffer B and 1 ml glucose-6-phosphate in a final volume of 2 ml as described previously (1) except the final buffer composition was maintained as buffer B. After 20 min at 37°, each mixture was supplemented to 4 mg/mL EG6 phosphatase and HEP were assayed by incubation at 100,000 rpm for 45 min. The pellets were resuspended in buffer B and aliquots were taken for determination of latency (s) and latency of glucose-6-phosphatase activity before and after supplementing to 0.4 mM sodium dodecyl sulfate.
The characteristics of liver glucose-6-phosphatase in the envelope of isolated nuclei and microsomes are identical.

W J Arion, L O Schulz, A J Lange, J N Telford and H E Walls

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