Evidence for Two Types of Rat Liver Microsomes with Differing Permeability to Glucose and Other Small Molecules*

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Radioisotope flux measurements using Millipore filtration revealed two populations of rat liver microsomes designated type A and B. Type A and B vesicles are similar in that both are essentially impermeable to sucrose yet permeable to Cl⁻. About 70% of the microsomes (type A) are permeable to D-glucose, L-glucose, 2-deoxy-D-glucose, D-mannose, D-mannitol, uridine, glycine, L-leucine, choline₄, Tris⁺, RB⁻, K⁺, and Na⁺. Other solutes such as D-glucosamine⁻, D-glucosamine⁻, N-acetyl-D-glucosamine, L-glutamate, L-lysine, sulfate⁻, oxalate⁻, and phosphate anions transverse type A vesicles with an intermediate rate. All of the above solutes except Cl⁻ pass with a comparatively slow rate the remaining 30% type B vesicles. Both type A and B microsomes are relatively impermeable to glucose 6-phosphate and related monophosphates. Membrane potential measurements using liver microsomes and control membrane vesicles derived from rabbit skeletal muscle sarcoplasmic reticulum indicated that type A liver microsomes, despite being permeable to K⁺ and Na⁺, either lack or contain only a small number of highly conducting K⁺ and Na⁺ structures, such as the K,Na channel of sarcoplasmic reticulum. Treatment with the anion transport inhibitor 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid lowered the permeability of type A vesicles to several uncharged and negatively charged solutes including D-glucose and glucocamine⁻. These results suggest that a large fraction of liver microsomes is rendered permeable to various biologically relevant solutes and ions, perhaps through the presence of one or more channels with a maximal diameter of approximately 7–8 Å which select(s) against solutes on the basis of their size and charge.

Several membrane permeation and transport systems are associated with the endoplasmic reticulum of liver cells. A calcium-sequestering system analogous to although less active than the well studied sarcoplasmic reticulum Ca⁺⁺-transport ATPase of striated muscle has been demonstrated in rat liver microsomes (Moore et al., 1975). Evidence has been presented that glucose-6-P hydrolysis is mediated by a two-component system consisting of a specific glucose-6-P permease which transfers glucose-6-P across the endoplasmic reticulum membrane and a non-specific phosphohydrolase-phosphotransferase which is localized on the luminal side of the membrane (Ballas and Arion, 1977). Polypeptide and saccharide transport proteins may be involved in the synthesis of structural and enzymatic membrane and/or extracellular proteins. A NH₂-terminal extension of about 20 relatively hydrophobic amino acids is believed to direct the growing polypeptide chain into the endoplasmic reticulum lumen (Blobel and Dobberstein, 1975), possibly via a polypeptide-permeable structure. In the case of N-glycosidically linked oligosaccharides, a dolichyl diphasphate lipid intermediate containing N-acetylglucosamine, glucose, and mannose has been implicated to serve as a direct precursor for the oligosaccharide chain of glycoproteins (Schainoni and Leloir, 1979). The lipid-saccharide intermediate is thought to be synthesized on the cytoplasmic side of the endoplasmic reticulum and after its movement across the membrane, to transfer the saccharide portion to the nascent polypeptide chain (Snider et al., 1980). Glucose and some of the mannose residues are subsequently released (Robbins et al., 1977; Tabas et al., 1978; Hunt et al., 1978). However, the mechanism(s) by which the lipid-saccharide intermediate and the released saccharides cross the membrane have not yet been resolved. Biosynthesis of O-glycosidically linked oligosaccharides is also poorly understood. In particular, it is not clear whether the saccharides are attached to the protein before or after the nascent polypeptide chain has been transferred across the membrane. In either case, it may be speculated that the endoplasmic reticulum membrane contains structures which facilitate movement of hydrophilic saccharides across the membrane.

In the present study, we have investigated the nature of the impermeability barrier formed by rat liver microsomes. We found that rat liver microsomes consist of two types of vesicles which differ in their permeability to various biologically relevant solutes. One group of vesicles (designated type A) is permeable to glucose and certain other small solutes, while the second type (type B) is relatively impermeable to these compounds.

MATERIALS AND METHODS

Reagents—Radioisotopes were obtained from the following sources: D-[U-¹⁴C]glucose, L-[6-³H]glucosamine, N-acetyl-d-[¹⁴C]glucosamine, L-[4,5-²H]lysine, and [5-³H]uridine from Amersham, Arlington Heights, IL; D-[U-¹³C]glucose from ICN Pharmaceuticals, Irvine, CA; Cl⁻, RB⁺, Na⁺, [methyl-¹³C]choline, [fructose-¹³C]sucrose, L-[¹³C]H-glucose, L-[¹³C]H-galactose, D-[¹³C]H-mannose, D-[¹³C]H-mannitol, L-[³H]glutamate, and L-[4,5-³H]H]leucine from New England Nuclear, Boston, MA. 4,4'-disothiocyanostilbene-2,2'-disulfonic acid was purchased from Pierce, Rockford, IL. The fluorescent dye 3,3'-dipentyl-2,2'-oxacarbocyanine was the generous gift of Dr. Alan S. Waggoner (Amherst College, Amherst, MA). Other reagents used were of reagent grade.

Isolation of Membranes—Microsomal membrane fractions derived from liver were prepared as described by Meissner and Allen (1979).

* The abbreviations used are: DIDS, 4,4'-disothiocyanostilbene-2,2'-disulfonic acid; dO-C₃(3), 3,3'-dipentyl-2,2'-oxacarbocyanine iodide; Pipes, 1,4-piperazinediethanesulfonic acid.
from rat liver were prepared as follows. A rat fed ad libitum and weighing about 200 g was decapitated using a guillotine. The liver was rapidly excised, placed into ice-cold 0.25 M sucrose, and cut into small pieces. After transfer into 5 volumes of 0.25 M sucrose and 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes) (pH 7.5), the tissue pieces were homogenized at 0 °C using a smoothwalled Potter-Elvehjem homogenizer with a Type A pestle (A. H. Thomas & Co., Philadelphia, PA). The homogenate was centrifuged for 20 min at 10,000 rpm (7,800 × gav) in a Beckman type 35 rotor. A microsomal fraction was obtained from the supernatant by centrifugation for 60 min at 35,000 rpm (100,000 × gav) in a Beckman type 42 rotor. The upper half of the pellets was resuspended in 100 ml of 400 mM sucrose and 5 mM K/Pipes (pH 7) and recentrifuged. The pellet (10–15 mg of protein/g of liver) was resuspended in a small volume of 400 mM sucrose and 5 mM K/Pipes (pH 7).

"Smooth" and "rough" microsomal fractions were prepared with the CsCl aggregation technique of Dalner (1963). To the 7,800 × g supernatant fraction (see above) was added 1 M CsCl to a final concentration of 15 mM. Eight milliliters of this mixture were layered over 5 ml of 1.3 M sucrose containing 15 mM CsCl. Centrifugation for 135 min at 55,000 rpm (200,000 × gav) in a Beckman type 75 rotor yielded a translucent, reddish pellet ("rough" microsomal fraction) and a well-defined band at the gradient interface ("smooth" microsomal fraction). Membranes present at the 0.25/1.3 M sucrose interface were diluted with 20 volumes of 400 mM sucrose and 5 mM K/Pipes (pH 7). The pellet fraction was resuspended in the same buffer. Both membrane suspensions were recentrifuged and resuspended (10–15 mg of protein/ml) of 400 mM sucrose and 5 mM K/Pipes (pH 7). Microsomes were quickly frozen and stored at −65 °C before use.

"Intermediate" density rabbit skeletal muscle sarcoplasmic reticulum vesicles used in this study have been characterized previously (Meissner and Young, 1980). The polarity of membrane potentials was determined by the use of the fluorescent dye diO-C12(3) under conditions similar to those used in the present study (Meissner and Young, 1980). The polarity of membrane potentials was reported according to standard convention, that is, reference (ground) is extravascular. Fluorescence assays were carried out at 15 °C under stirring in a Farrand model 601 fluorometer. Excitation was at 470 nm and emission was recorded at 495 nm. Slits used resulted in a half-band width of 2.5 nm. Vesicle concentrations (approximately 25 μg of protein/ml) were used which produced negligible perturbation of the fluorescence emission during dilution with incubation medium.

**RESULTS**

**Properties of Liver Microsomal Fractions**—Electron micrographs of unfractionated rat liver microsomes and the two sucrose gradient fractions revealed closed membranous vesicles with diameters ranging from approximately 0.1–0.2 μm (Fig. 1). Membranes sedimenting through the 1.3 M sucrose layer of the gradient had a rough, granular appearance, suggesting the presence of ribosomes bound to the external, cytoplasmic surface. Membranes collected from the 1.3/1.3 M sucrose interface of the gradient lacked membrane-bound ribosomes. The more heterogeneous appearance of the interface fraction reflected the fact that it was likely derived in part from organelles other than smooth endoplasmic reticulum. Enzymatic analysis indicated that unfractionated, rough and smooth microsomes hydrolyzed glucose-6-P, an enzymatic activity typically associated with endoplasmic reticulum of rat liver (Table I). The fractions contained small quantities of inner mitochondrial membranes, plasma membranes, and lysosomes, as indicated by their low succinate-cytochrome c reductase, 5'-nucleotidase, and acid phosphatase activities, respectively.

**Isofree Flux Experiments**—The permeability of liver microsomes to [H]sucrose and d-[14C]glucose was determined by Millipore filtration as described under "Materials and Methods." The two radioactive compounds (at a concentration of 1 μCi/ml) were incubated with microsomal membranes in the presence of [3H]sucrose and [14C]glucose. Radioactivity filtration at time intervals ranging from 1/2–4 min. Radioactivity filters with the vesicles were then filtered and washed with an unlabeled iso-osmolar medium and were collected on Millipore filters at time intervals ranging from 1/2–4 min. Radioactivity filters with the vesicles was determined. As shown in Fig. 2, microsomes possessed a permeability barrier for [H]sucrose and d-[14C]glucose. Reasonably straight lines were obtained suggesting that radioisotope efflux may be approximated by first order kinetics. Comparison of the slopes indicated that the efflux rate of d-glucose (t_{1/2} = 2–3 min) between 30 s and 4 min was somewhat greater than that of sucrose (t_{1/2} ≥ 5 min). A striking difference was that the apparent d-[14C]glucose spaces amounted to only a fraction of the [H]sucrose spaces. One possible, although not likely, explanation for this difference was that an incubation time of 1 h was sufficient to equilibrate [H]sucrose but not d-[14C]glucose across the vesicle membranes. We tested for this possibility by incubating microsomes for times ranging from 30 min to 5 h. Vesicles were then filtered and washed with a radioactive isotope space for [H]sucrose and d-[14C]glucose. From the 0.25/1.3 M sucrose interface of the gradient lacked membrane-bound ribosomes. The more heterogeneous appearance of the interface fraction reflected the fact that it was likely derived in part from organelles other than smooth endoplasmic reticulum. Enzymatic analysis indicated that unfractionated, rough and smooth microsomes hydrolyzed glucose-6-P, an enzymatic activity typically associated with endoplasmic reticulum of rat liver (Table I). The fractions contained small quantities of inner mitochondrial membranes, plasma membranes, and lysosomes, as indicated by their low succinate-cytochrome c reductase, 5'-nucleotidase, and acid phosphatase activities, respectively.

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Permeability of Liver Microsomes

Fig. 1. Electron micrographs of liver microsomal fractions. Samples were fixed with 2.5% glutaraldehyde and 0.5% tannic acid in 0.3 M sucrose, pH 7.2, for 15 min in ice and 45 min at 23 °C and sedimented. Pellets were postfixed with 1% OsO4, embedded, sectioned, and stained as previously described (Malouf and Meissner, 1979). A,B,C, unfractionated, rough, smooth liver microsomal fractions, respectively: × 30,300.

plots (Fig. 3) by two nearly straight and parallel lines suggesting that the decrease in isotope space was of first order and independent of the isotope tested.

A similar difference in sucrose and d-glucose isotope spaces was obtained when 10 mM sucrose or glucose was omitted from the release medium or when incubation and release media were used that contained 400 mosM sucrose or D-glucose (see below). In another control, we found that rabbit skeletal muscle sarcoplasmic reticulum vesicles (Meissner, 1975) had essentially identical [3H]sucrose and D-[14C]glucose spaces (2.9 μl/mg of protein).

Table I shows the sucrose and glucose isotope spaces determined for unfractionated, rough and smooth liver microsomal fractions. In unfractionated and rough fractions, D-[14C]glucose space accounted for about 25–35% of the [3H]sucrose space. In the smooth fraction, about one-half of the vesicles were relatively impermeable to glucose. [3H]sucrose and D-[14C]glucose efflux rates for rough and smooth vesicle fractions were essentially identical with those shown for unfractionated liver microsomes in Fig. 2.

Our explanation for the different isotope spaces of sucrose and D-glucose is that liver microsomes consist of vesicles which differ in their permeability to sucrose and D-glucose. Between 50 and 75% of the vesicles release D-[14C]glucose within 20–30 s, i.e. before the first time point was taken. These vesicles (designated type A) appear, therefore, permeable to glucose, presumably because they contain a mechanism that facilitates the release of D-glucose. The remaining 25–50% of the vesicles (designated type B) had a low D-[14C]glucose permeation rate similar to that of [3H]sucrose and subsequently seem to lack a permeation system for D-glucose.

The apparent isotope spaces of liver microsomes to a variety of additional solutes were determined, as described for [3H]sucrose and D-[14C]glucose in Figs. 2 and 3. The related compounds L-glucose, 2-deoxy-D-glucose, D-mannose, and D-mannitol as well as the nucleoside uridine behaved like D-glucose in that essentially identical efflux rates and apparent isotope spaces were observed. Each of these uncharged solutes had an apparent isotope space of 0.85–1.0 μl/mg of protein which corresponded to 30–40% of the [3H]sucrose space (Table II). Similarly low apparent isotope spaces were measured for the univalent cations "Rb", "Na", and [3H]choline. After 30 s of dilution, the cations left the vesicles with a rate (t1/2 = 1–2 min) about twice as fast as D-glucose (cf. Fig. 2).
Properties of liver microsomal fractions

The unfractonated microsomal fraction represents material sedimenting between 7,800 × g and 100,000 × g. Smooth and rough microsomes sedimented at the 0.25 M/1.3 M sucrose interface and through the 1.3 M layer of the gradient, respectively. Enzymatic assays were carried out as described under "Materials and Methods." About one-third of the protein of rough microsomes is of ribosomal origin (Fleischer and Kervina, 1974). Accordingly, the specific activity of glucose-6-P phosphohydrodrolase in rough microsomes corrected to membrane protein (minus ribosomes) is 66, as compared to 49 for smooth microsomes. ["H]Sucrose and D-[^14C]glucose spaces were obtained by extrapolating the efflux rates and isotope spaces back to zero time as indicated by the broken lines in Figs. 2 and 3. "Cl- spaces were determined by measuring "Cl- influx and efflux in a medium containing 10 mM "Cl, as described in Figs. 2 and 3.

![FIG. 2 (left). Measurement of ["H]sucrose and D-[^14C]glucose efflux rates and isotope spaces. Unfractionated liver microsomes (10 mg of protein/ml) were incubated for 1 h at 23 °C in a medium containing 400 mosM K/Pipes (pH 7), 10 mM ["H]sucrose, and 10 mM D-[^14C]glucose. Vessicles were then diluted 500-fold into an unlabeled medium of identical composition at 23 °C. Aliquots of 0.5 ml were placed on Millipore filters, rinsed, and the amounts of radioactivity remaining with the vesicles on the filters were determined. ["H]Sucrose and D-[^14C]glucose spaces extrapolated back to zero time corresponded to 2.1 and 0.8 µl/mg protein, respectively.

![FIG. 3 (right). Effect of incubation time on ["H]sucrose and D-[^14C]glucose spaces of liver microsomes. Unfractionated microsomes were incubated at 23 °C for the indicated times in a medium containing 400 mosM K/Pipes (pH 7), 10 mM ["H]sucrose, and 10 mM D-[^14C]glucose. Vessicles were then diluted and processed as described in Fig. 2. ["H]sucrose and D-[^14C]glucose spaces were obtained by back extrapolation to the time of vesicle dilution (cf. Fig. 2).

D-[^14H]Glutamate and D-[^14H]glucosamine" spaces were comparable to the ["H]sucrose space. N-acetyl-D-glucosamine displayed an apparent isotope space intermediate between that of D-glucose and the two charged species glutamate and glucosamine. Table II. Apparent isotope spaces of liver microsomes to ["H]sucrose and other solutes

Isotope spaces were determined by measuring isotope influx and efflux across unfractionated liver microsomes as described in Figs. 2 and 3. Efflux rates and isotope spaces were extrapolated back to zero time, as indicated by the broken lines in Figs. 2 and 3. The incubation and dilution media contained 400 mosM K/Pipes (pH 7) and 10 mM concentration of the test solute. Data are the average of three or more determinations. S.E. = ± 15%.

| Radioisotope | Apparent isotope space | µl/mg protein | % ["H]sucrose space |
|--------------|-----------------------|---------------|---------------------|
| ["H]Sucrose  |                       | 2.5           | 100                 |
| ["H]Choline  |                       | 1.05          | 42                  |
| "Rb         |                       | 0.9           | 36                  |
| D-[^14C]Gluconate |               | 0.85          | 34                  |
| D-[^14H]Glucosamine |         | 0.95          | 38                  |
| N-Acetyl-D-[^14H]glucosamine | | 0.95          | 38                  |
| D-[^14C]Glucose |                 | 0.9           | 36                  |
| L-[^14H]Glucose |                 | 1.0           | 40                  |
| ["H]Mannose  |                       | 0.85          | 34                  |
| D-[^14H]Mannitol |                 | 0.9           | 36                  |
| ["H]Uridine  |                       | 0.9           | 36                  |
| D-[^14C]Leucine |                 | 1.25          | 50                  |
| L-[^14C]Glutamate |             | 2.6           | 104                 |
| L-[^12]Lysine |                       | 3.2           | 128                 |

Osmotic Swelling and Rupture Experiments—Above we suggested that liver microsomes consist of vesicles, some of which are readily permeable to D-glucose (type A) and some that are not (type B). Both types of vesicles were relatively impermeable to sucrose. One might expect then that dilution of sucrose-filled vesicles into glucose medium would generate an osmotic force in glucose-permeable vesicles due to rapid influx of solute and water. Vesicles will swell and disrupt which in turn would induce rapid release of sucrose thereby lowering internal osmotic pressure of the vesicles. On the other hand, vesicles with a glucose diffusion barrier comparable to that of sucrose should not disrupt and should, therefore, be able to retain their content for longer times. To test this hypothesis, osmotic behavior of vesicles filled with 400 mosM ["H]sucrose or D-[^14C]glucose was studied. In control experiments, vesicles were diluted into an unlabeled dilution medium with a com-
Sucrose and D-[14C]glucose efflux rates and spaces comparable to those in Fig. 2 were observed. In another control experiment, it was established that both type A and B were osmotically active. Dilution of the vesicles into a medium of low osmolality resulted in release of most, but not all, of the trapped [3H]sucrose and D-[14C]glucose (Fig. 4). The remaining radioactivity diffused across the vesicle membranes with a rate comparable to that observed under iso-osmolar conditions. Apparently, the vesicles reformed a permeability barrier; once enough sucrose and glucose was released to lower inter-

As a fast and slow efflux component was also distinguished when sucrose-filled vesicles were diluted into iso-osmolar glucose medium (Fig. 4). The amount of [3H]sucrose released was in accord with about 70% of the vesicles (type A) being readily permeable to glucose. Retention of about 30% of the [3H]sucrose suggested that the remaining vesicles (type B) were relatively impermeable to both sucrose and glucose, in agreement with the isotope flux studies.

The permeability of type A and B vesicles to additional solutes was analyzed by taking advantage of their differing glucose permeability. Vesicles were filled with 390 mosm [3H]sucrose and 10 mosm D-[14C]glucose and diluted into isoosmolar media containing the test compound (Table III). Transfer of the vesicles into a medium of low osmolality or into D-glucose medium demonstrated that rupture of type A and B vesicles or type A vesicles, respectively, is observable as [3H]sucrose and D-[14C]glucose release (Experiments 1–3 of Table III). Most of the trapped D-[1H] and [14C]radioactivity was also released when vesicles were transferred into a KCl medium containing the K+-selective ionophore valinomycin. As shown in Fig. 4, for [3H]sucrose- or D-[14C]glucose-filled vesicles diluted into H2O, a rapid initial release phase was followed by normal, slow [3H]sucrose and D-[14C]glucose leakage rates. Since the generation of an osmotic force required the inward flow of Tris+ or choline+ along with C1- and water rapidly entered the vesicles resulting in their rupture and the release of their contents. Thus, the osmotic swelling and rupture experiment confirmed isotope flux measurements (Table I) which had also indicated that most of liver microsomes are intrinsically permeable to Cl-. Omission of valinomycin from the KCl release medium or dilution into NaC1, choline/Cl, or Tris/Cl medium resulted in a reduction of the initial burst of [3H]sucrose and D-[14C]glucose release. Nearly optimal D-[14C]glucose spaces were observed when vesicles were diluted into choline/Cl or Tris/Cl media. In choline/Cl and Tris/Cl media, amounts of [3H]sucrose retained by the vesicles increased to a similar extent as those for D-[14C]glucose. Similar D-[14C]glucose efflux rates and isotope spaces were obtained in the above experiments when microsomes were used which contained 400 mosm D-[14C]glucose instead of 390 mosm [3H]sucrose and 10 mosm D-[14C]glucose (not shown). We interpret these results to show that type A vesicles are permeable to D-glucose as well as to K+, Na+, Tris+, and choline+. Glucose-impermeable (type B) vesicles appeared, on the other hand, to be relatively impermeable to Tris+ and choline+. These vesicles thereby avoided massive inflow of Tris+ or choline+ along with CI- and water and subsequent rupture. Type B vesicles appeared also to form a permeability barrier to K+ and Na+, although at a reduced level in comparison with Tris+ and choline+.

In the additional compounds listed in Table III, essentially two groups of solutes could be distinguished, D-mannose, D-mannitol, uridine, and glycine behaved like D-glucose in that they caused a large initial burst of [3H]sucrose release. Amounts of D-[14C]glucose remaining with the vesicles were comparable to those found in the D-glucose control medium or were actually greater than in the control medium, as found for uridine or D-mannose. The reason for the increased

**Table III**

| Composition of dilution medium | [3H]Sucrose space | % Sucrose space | D-[14C]Glucose space | % D-Glucose space |
|-------------------------------|------------------|----------------|---------------------|------------------|
|                                | µl/mg protein    |                | µl/mg protein       |                  |
| Sucreose                      | 1.75             | 100            | 0.35                | 70               |
| d-Glucose                     | 0.49             | 28             | 0.50                | 100              |
| H2O                           | 0.21             | 12             | 0.17                | 54               |
| KCl + Val                     | 0.17             | 10             | 0.15                | 30               |
| KCl                           | 0.26             | 15             | 0.25                | 50               |
| NaCl                          | 0.23             | 13             | 0.32                | 64               |
| Tris/HC                       | 0.35             | 20             | 0.45                | 90               |
| Choline/Cl                    | 0.44             | 25             | 0.43                | 86               |
| MgCl                          | 1.40             | 80             | 0.65                | 100              |
| Pipes (K + Val)               | 1.45             | 83             | 0.48                | 96               |
| d-Glucosamine/HC              | 1.05             | 60             | 0.40                | 80               |
| N-Acetyl-D-glucosamine        | 0.70             | 40             | 0.50                | 100              |
| d-Glucose                     | 0.86             | 49             | 0.43                | 86               |
| d-Mannitol                    | 0.49             | 28             | 0.75                | 133              |
| Uridine                       | 0.51             | 29             | 0.52                | 104              |
| Glycine                       | 0.45             | 26             | 0.67                | 134              |
| L-Glutamate (K + Val)         | 1.65             | 60             | 0.47                | 94               |
| L-Lysine (HC)                 | 1.15             | 66             | 0.55                | 110              |
amounts of d-[14C]glucose in the presence of the two latter solutes is not clear at present. The second group of components which included K/glucuronate (Fig. 4) was intermediately effective in increasing the initial [1.4H]sucrose leakage rate. The chloride salts of Mg2+, lysine, and glucosamine as well as N-acetyl-D-glucosamine and K/glutamate behaved like K/glucuronate in that reduced initial bursts of [1.4H]sucrose release were generated on dilution into media containing these compounds (Table III). Levels of d-[14C]glucose remaining with the vesicles 30 s after dilution were similar to that of the control, i.e. when vesicles were diluted into D-glucose medium.

Assuming that influx of salts is largely determined by the slower of the two penetrating ions present in the test medium, the [1.4H]sucrose and d-[14C]glucose release experiments suggested significant differences between the permeability barriers formed by type A and B vesicles. Glucose-permeable (type A) vesicles appeared to be readily permeable to a variety of small neutral and charged molecules. Glucose-impermeable (type B) vesicles differed from type A vesicles in that the same solutes, with the exception of K+, Na+, and Cl−, permeated across their membranes similarly slowly as sucrose. The results of osmotic swelling and rupture experiments were in reasonable agreement with the isotope flux measurements.

Membrane Potential Experiments—Previous isotope and membrane potential measurements have shown that fragmented rabbit skeletal muscle sarcoplasmic reticulum consists of two vesicle populations, one of which contains a K,Na channel and a second one that does not (McKinley and Meissner, 1977, 1978). The ratio of (K+,Na+)-permeable and -impermeable vesicles was approximately 2:1. Both types of vesicles were relatively impermeable to Ca2+, Mg2+, and larger univalent ions such as glucuronate, Pipes, choline, or Tris−. The fluorescent dye cyanine was used to visualize vesicles which could generate a K+-induced membrane potential (negative inside). Vesicles elicited no change in fluorescence when diluted into K or Na/Pipes medium, indicating that no membrane potential was created (McKinley and Meissner, 1978). However, when vesicles were diluted into Mg, Ca, or Tris/Pipes medium in the absence of valinomycin, fluorescence signals of intermediate size were detected. Under these conditions, only the (K+,Na+)-permeable vesicle fraction formed a negative membrane potential. The absence of a potential in (K+,Na+)-impermeable vesicles was due to the similarly low permeability of K+, Mg2+, Ca2+, and Tris−. Addition of valinomycin to the Mg2+, Ca2+, or Tris− medium made (K+,Na+)-impermeable vesicles permeable to K+ and resulted in a maximal dye signal since it enabled formation of a membrane potential in the entire vesicle population, i.e. (K+,Na+)-permeable and -impermeable vesicles. On the other hand, a negative potential was exclusively formed in (K+,Na+)-impermeable vesicles by transferring the entire sarcoplasmic reticular vesicle population from K+ to Na+ medium containing valinomycin. Under these conditions, no membrane potential was formed in (K+,Na+)-impermeable vesicles. Because of the presence of the K,Na channel, these vesicles rapidly exchanged all of their K+ for Na+ within 1–2 s, the experimental limit of detection. Within 1 h, fluorescence signals returned to their original values. This gradual reduction in membrane potential was likely due to slow inward movement of the extravascular cation (Mg2+, Ca2+, Tris−, or Na+) and the eventual dissipation of the K+ gradients.

Liver microsomes showed a similar, although not identical, behavior when transferred from K/Pipes to Mg or Ca/Pipes medium in the presence or absence of valinomycin (Fig. 5B). Liver microsomes and sarcoplasmic reticulum vesicles differed in their K+ permeability in that the initial fluorescence decreases and apparent isotope spaces assigned to type A and type B vesicles were about the same. Liver microsomes also generated a fluorescent signal of intermediate size when transferred from Na or Tris to Mg or Ca/Pipes medium (not shown). Thus, in agreement with the osmotic rupture experiments (cf. Table III), fluorescent measurements indicated that a substantial portion of the microsomes was more permeable to K+, Na+, and Tris− than to Mg2+ or Ca2+.

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changes in Mg or Ca/Pipes medium were appreciably slower for liver microsomes (20–30 s) than for sarcoplasmic reticulum vesicles (~2 s). Despite their preferential K⁺ permeability, a majority of liver microsomes appeared, therefore, to lack an ion-conducting structure for K⁺ such as the KNa channel which renders two-thirds of sarcoplasmic reticulum vesicles highly permeable to K⁺.

Absence of a “channel” rendering liver microsomal vesicles highly permeable to K⁺ and Na⁺ was supported by the following two observations. First, transfer of microsomes from K⁺ to Tris⁺ medium did not elicit an appreciable dye response, suggesting that most of the microsomes did not form a potential. Second, liver microsomes rendered permeable to K⁺ by valinomycin elicited a similar dye signal when transferred to Na⁺ or Tris⁺ medium. The rapid collapse of membrane potentials in Na⁺ or Tris⁺ medium as well as the reduced magnitude of dye signals in Na⁺ or Tris⁺ medium, as compared to those in Mg²⁺ or Ca²⁺ medium, were in accord with the differential permeability of liver microsomes to these cations (cf. Table III).

In summary, membrane potential experiments support our contention that liver microsomes are more permeable to K⁺, Na⁺, and Tris⁺ than to Mg²⁺ or Ca²⁺. However, the majority of liver microsomes appeared to lack a highly conducting K⁺ and Na⁺ structure, such as the KNa channel of sarcoplasmic reticulum.

### Permeability of Liver Microsomes to Glucose-6-P

In osmotic swelling and rupture experiments, liver microsomes were routinely kept for 90 min at 23 °C in order to allow [³H]sucrose and [¹⁴C]glucose to diffuse across vesicle membranes. Table IV shows that latency of mannose-6-P phosphohydrolase activity (Arion et al., 1972) was maintained, suggesting that liver microsomes retained a permeability barrier to mannose-6-P. In addition, nearly identical glucose-6-P phosphohydrolase activities by intact or disrupted microsomes before and after incubation indicated that glucose-6-P transport and phosphohydrolase activities were not appreciably impaired by preincubation for 90 min at 23 °C. Glucose-6-P phosphohydrolase activity by intact microsomes was inhibited by the anion transport inhibitor DIDS, as previously reported (Zoccoli and Karnovsky, 1980) (Table IV). Disruption of the permeability barrier by treating membranes with deoxycholate reversed inhibition of glucose-6-P phosphohydrolase activity. These results were in accord with previous suggestions that blockage of glucose-6-P permeation will limit glucose-6-P access to its hydrolytic site on the cisternal membrane surface (Arion et al., 1972; Gold and Widnell, 1976; Ballas and Arion, 1977; Zoccoli and Karnovsky, 1980). Kinetic parameters of glucose-6-P phosphohydrolase in intact and deoxycholate treated microsomes were determined under conditions similar to those used in isotope flux experiments using varying concentrations of glucose-6-P (0.5–10 mM). The double reciprocal Lineweaver-Burk plots of the data yielded two straight lines (not shown). From the intercepts with the abscissa and ordinate, apparent $K_m$ values of 6.1 and 2.9 mM and $V_{max}$ values of 0.10 and 0.15 μmol/mg of protein/min were obtained for intact and detergent-disrupted microsomes, respectively. Corresponding $K_m$ and $V_{max}$ values for mannose-6-P phosphohydrolase activity by deoxycholate treated microsomes were 6.1 mM and 0.15 μmol/mg of protein/min, respectively (Table IV). The increase in $V_{max}$ from 0.10–0.15 mol/mg of protein/min raised the possibility that one-third of the intact microsomes were not capable of glucose-6-P hydrolysis because of their lack of an efficient glucose-6-P permeation system. An alternative explanation would be that a change in membrane structure during detergent treatment resulted in an increased turnover rate of glucose-6-P hydrolysis.

### Table IV

| Kinetic parameters and effects of reagents on glucose-6-P and mannose-6-P phosphohydrolase activities of liver microsomes |
|---|
| **Experiment 1:** Unfractionated liver microsomes (10 mg of protein/ml) present in 5 mM K/PIPES buffer (pH 7) containing 400 mM sucrose or glucose were pretreated by incubation (i) for 90 min at 23 °C, or (ii) with 1 mM DIDS for 5 min at 30 °C. Treated samples were then either directly diluted into the standard glucose-6-P phosphohydrolase assay medium (cf. “Materials and Methods”) or were first disrupted before the assay by transferring them into standard glucose-6-P phosphohydrolase assay medium containing microsomes at a protein concentration of 2 mg/ml and 0.13% deoxycholate (DOC). Data are the average of four determinations. S.E. = ±10%. Experiment 2: kinetic parameters of glucose-6-P and mannose-6-P phosphohydrolase activities were determined from Lineweaver-Burk plots. Dependence of the enzymatic activities of intact and deoxycholate-treated (see above) liver microsomes on their respective substrate concentrations (0.5–10 mM) was determined at 23 °C in a 5 mM K/PIPES buffer (pH 7) containing 400 mM KCl. |
| **Glucose-6-P phosphohydrolase** | **Mannose-6-P phosphohydrolase** |
| --- | --- | --- |
| $V_{max}$ (nmol/mg protein/min) | 0.10 | 0.15 |
| $K_m$ (mM) | 6.1 | 2.9 |

### Experiment 1

| Pretreatment of microsomes | Glucose-6-P phosphohydrolase | Mannose-6-P phosphohydrolase |
|---|---|---|
| None (control) | 37 | 95 | 5 | 76 |
| 90 min at 23 °C | 30 | 90 | 5 | 76 |
| DIDS (1 mM) | 5 | 73 | --- | --- |

### Experiment 2

**Kinetic parameters**

| Parameter | Value |
|---|---|
| $K_m$ (mM) | 6.1 |
| $V_{max}$ (μmol/mg protein/min) | 0.10 |

A significant reduction in the initial burst of [³H]sucrose and an increase in the apparent $t_{1/2}$ of [¹⁴C]glucose vesicle space were seen on transfer of DIDS-treated microsomes into D-
The microsomes were then diluted 500-fold at 23 °C into 5 mM K/Pipes buffer (pH 7) containing the indicated solute and no or 0.5 µM valinomycin (Val). Isotope space = $[^{3}H]$sucrose and D-$[^{14}C]$glucose vesicle spaces were noted in media containing lysine/HCl, choline/C1, or KC1 plus valinomycin. The observed increase in sucrose and D-glucose isotope spaces raised the possibility that treatment with DIDS significantly lowered the permeability of liver microsomes to certain uncharged and negatively charged solutes such as D-glucose and gluconate.

**TABLE V**

| Composition of dilution medium | $[^{3}H]$Sucrose | $[^{14}C]$Glucose |
|-------------------------------|------------------|------------------|
|                               | -DIDS +DIDS      | -DIDS +DIDS      |
| Sucrose                       | 1.65 1.85        | 0.22 0.30        |
| D-Glucose                     | 0.45 0.75        | 0.40 0.63        |
| D-Mannitol                    | 0.43 0.66        | 0.38 0.58        |
| KC1 (+Val)                    | 0.22 0.22        | 0.12 0.13        |
| Choline/Cl                    | 0.38 0.36        | 0.25 0.26        |
| Glucose-6-P (K + Val)         | 1.35 1.30        | 0.30 0.42        |
| Mannose-6-P (K + Val)         | 1.45 1.35        | 0.35 0.52        |
| Glucose-1-P (K + Val)         | 1.45 1.45        | 0.35 0.52        |
| Uridine-6-P (K + Val)         | 1.45 1.35        | 0.35 0.52        |
| Phosphate (K + Val)           | 0.8 1.2          | 0.32 0.51        |
| Sulfate (K + Val)             | 1.0 1.3          | 0.36            |
| Oxalate (K + Val)             | 0.95            | 0.37            |
| D-Glucosamine                 | 0.85 1.45        | 0.30 0.51        |
| N-Acetyl-d-glucosamine        | 0.75 1.4         | 0.40 0.74        |
| L-Glutamate (K + Val)         | 0.90 1.25        | 0.34 0.51        |
| L-Lysine/HCl                  | 1.1 0.8          | 0.32 0.32        |

**DISCUSSION**

This study has shown that rat liver microsomes are composed of two types of vesicles which differ in their permeability to various biologically relevant solutes including D-glucose. Vescicles referred to as type A are relatively impermeable to sucrose but are readily permeable to D-glucose. In contrast, type B microsomes are relatively impermeable to both sucrose and D-glucose. Observation that type A but not type B vesicles are permeable to a variety of additional solutes suggests that type A microsomes contain permeases or “channels” which facilitate movement of D-glucose and other small molecules.

The permeability of rat liver microsomes has been previously investigated. Nilsson et al. (1973) found that uncharged solutes with a molecular weight of up to 600 including D-glucose and sucrose equilibrated across the microsomal membranes within 2 h, the time used to pellet microsomes by centrifugation. By contrast, microsomes appeared to be impermeable to charged molecules with a molecular weight as low as 90. Ballas and Arion (1977) used the technique of centrifugal transfer through a layer of silicone oil to separate rapidly Ca2+-aggregated microsomes from bulk medium. Microsomes were found to be permeable to glycerol, D-glucose, D-mannose, and glucose-6-P but not to polyethylene glycol, M, = 4000). Mannose-6-P was also found to penetrate the microsomal membranes, although at a slower rate than glucose-6-P. Our studies indicated that the majority of microsomes were appreciably less permeable to glucose-6-P and mannose-6-P than to D-glucose or D-mannose. Evidence for low permeability of intact microsomes to larger anions was previously provided by the observation that EDTA solubilized only a small portion of lead phosphate precipitates formed within the microsomal vesicle space by incubation in the presence of glucose-6-P and Pb2+ (Gold and Widnell, 1976). The previous studies did not describe the presence of two types of vesicles which differ in their permeability to small solutes.

The most economical explanation for the permeability of type A vesicles to various solutes would be that these vesicles contain a single permease or "channel" which selects against solutes on the basis of their size and charge. The three uncharged solutes D-glucose, N-acetyl-D-glucosamine, and sucrose passed with a decreasing permeation rate across the membranes of type A microsomes. Taking into consideration the van der Waal’s dimensions of these 3 molecules, solute selectivity of liver microsomes can be explained in geometrical terms by assuming that type A vesicles contain a channel with a diameter of approximately 7-8 Å. Accordingly, solutes with a minimal cross-section of less than 7-8 Å such as D-glucose would be able to pass through the pore, while solutes with a cross-section exceeding 7-8 Å such as sucrose would be retarded. Challenging this hypothesis was our observation that the permeability of type A membranes to both glucosamine and gluconate was lower than that to D-glucose despite the fact that both molecules are small enough to fit through a pore with a diameter of 7-8 Å. It seems, therefore, that, in...
Permeability of Liver Microsomes

addition to size, overall charge is important in determining the permeation rate of a molecule through the channel. Selectivity against charged molecules seemed to become more pronounced with an increase in charge density, as evidenced by the limited permeability of type A microsomes to the relatively small divalent ions Mg"++, sulfate"-, or oxalate"-. In support of a single channel structure was that DIDS treatment decreased permeability of type A microsomes to several solutes including d-glucose and gluconate-. At present, it cannot be ruled out, however, that liver microsomes contain more than one permeation system. In fact, DIDS treatment did not significantly reduce membrane permeability to positively charged molecules such as choline+ or lysine+. Enzyme competition (Ballas and Arion, 1977) and DIDS labeling (Zoccoli and Karnovsky, 1980) studies have raised the possibility that distinct permeation systems exist for glucose-6-P, P, and P,P,, and one-channel permeation system because our experimental approaches were limited. For example, we could not determine the exact permeation rate of two fast or slow moving solutes such as d-glucose and glucose-6-P and mannose-6-P. In agreement with previous reports (Ballas and Arion, 1977) enzymatic analysis indicated a faster permeation rate for glucose-6-P than mannose-6-P. The difference in the rates was because, in contrast to osmotic rupture experiments, enzyme assays could be carried out at a substrate concentration below the K, of the glucose-6-P permease thereby minimizing nonmediated permeation. On the other hand, we could clearly distinguish between readily and slowly permeating solutes such as d-glucose and sucrose. Estimating liver microsomes to have on an average a diameter of 0.15 pm (Fig. 1) and assuming sucrose permeation to be a first order process with a half-time of 5 min (Fig. 2), the sucrose permeability coefficient is calculated to be on the order of 10^-6 cm/s. For comparison, from enzymatic data of Table IV, a glucose-6-P permeability coefficient of about 10^-1 cm/s is calculated, assuming a substrate concentration of 0.16 x and glucose-6-P permeation to be the rate-limiting step. Minimal [14C]glucose leakage from sucrose-filled vesicles on transfer into glucose-6-P medium also suggested a similarly low permeation rate for glucose-6-P and sucrose. By contrast, complete release of d-[14C]glucose from type A vesicles within 20-30 s suggested that these vesicles were at least 20 times more permeable to glucose than to glucose-6-P or sucrose. Isotope flux, osmotic rupture, and membrane potential measurements all indicated that solute permeation was likely free rather than coupled to another solute.

Glucose-permeable, type A microsomes are not considered to be simply "leaky" vesicles since both types of vesicles formed a permeability barrier to sucrose, Mg++, and Pipes-. The presence of type A vesicles in rough and smooth liver microsomal fractions further suggests that permeases mediating the movement of various solutes are distributed over the entire reticulum structure. The existence of vesicles differing in their permeability properties in isolated membrane fractions may be explained by assuming that the in situ reticulum structure contains a limited number of permeable structures. Fragmentation of the reticulum during homogenization will, therefore, yield some vesicles which contain a channel and others that do not. In this regard, it is of interest that (K+,Na-) permeable and -impermeable vesicles with a ratio of about 2:1 have been isolated from skeletal muscle sarcoplasmic reticulum. Sonication and reconstitution experiments have led us to suggest that formation of (K+,Na-)-impermeable sarcoplasmic reticulum vesicles is due to a limited number of randomly distributed K,Na channels, approximately 50/µm^3 (McKinley and Meissner, 1978; Young et al., 1981).

Despite the presence of (K+,Na-)-permeable and -impermeable vesicles in both the rat liver and rabbit skeletal muscle microsomal fractions, several important differences were noted to exist between the cation permeability of the two membranes. The majority of sarcoplasmic reticulum vesicles appeared to be relatively impermeable to d-glucose as indicated by the similar d-glucose and sucrose isotope spaces and efflux rates. This would suggest that skeletal muscle sarcoplasmic reticulum either lacks or contains only a small number of d-glucose-permeable structures. By contrast, rat liver endoplasmic reticulum seems to lack a substantial number of structures which would render this membrane highly permeable to K+ and Na+, as is the case with sarcoplasmic reticulum. Another significant difference between these two membranes is that sarcoplasmic reticulum membranes contain a H+-permeable pathway, whereas liver microsomes appear to lack a pathway that renders them highly permeable to H+ (Meissner and Young, 1980).

Different permeability behavior of liver and skeletal muscle reticulum membranes is in accord with their different physiological functions. Sarcoplasmic reticulum controls skeletal muscle contraction and relaxation by rapidly releasing and sequestering Ca++. Appreciable Ca++ fluxes are possible because they can be compensated in charge by the counter movement of H+, K+, and Na+ via the H+ and K,Na channels of sarcoplasmic reticulum (Meissner, 1981). Because of the absence of highly active Ca++-releasing or -sequestering transport systems in liver endoplasmic reticulum, specific high capacity permeation systems for H+, K+, and Na+ appear not to be required for this membrane. A major function of endoplasmic reticulum of liver is the biosynthesis of lipids and proteins. An NH2-terminal extension of about 20 relatively hydrophobic amino acids is cleaved from the growing polypeptide chain after transfer across the reticulum membrane. While the fate of this "leader" sequence is not known, its degradation inside the endoplasmic reticulum cisternae is conceivable. Similarly, glucose residues are released during oligosaccharide processing inside the reticulum cisternae. Glucose-6-P hydrolysis by endoplasmic reticulum requires that glucose-6-P, P, and glucose cross the reticulum membrane. One possible function of the channel(s) present in type A vesicles is to facilitate these processes by mediating the rapid inward and outward movement of substrates and reactants. Whether the d-glucose-permeable channel has any direct role in directing the growing polypeptide chain or oligosaccharide precursors across the reticulum membrane remains to be established.

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REFERENCES

Ballas, L. M., and Arion, W. J. (1977) J. Biol. Chem. 252, 8512-8518
Ballest, G., and Dobberstein, B. (1975) J. Cell Biol. 67, 835-851
Bout, P. S., and Toribara, T. Y. (1956) Anal. Chem. 28, 1756-1758
Daftner, G. (1963) Acta Pathol. Microbiol. Scand. Suppl. 166
Fleisher, S., and Fleisher, B. (1967) Methods Enzymol. 10, 406-433
Fleischer, S., and Kervina, M. (1974; Methods Enzymol. 31, 5-61
Gold, G., and Widnell, C. C. (1976) J. Biol. Chem. 251, 1035-1041
Hunt, L. A., Echison, J. R., and Summers, D. F. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 764-768
Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275

McKinley, O. H., and Meissner, L. (1978) Methods Enzymol. 47, 2551-2557
Meissner, L., and Young, W. (1980).
Malouf, N. N., and Meissner, G. (1979) *Exp. Cell Res.* **122**, 233-250
McKinley, D., and Meissner, G. (1977) *FEBS Lett.* **82**, 47-50
McKinley, D., and Meissner, G. (1978) *J. Membr. Biol.* **44**, 159-186
Meissner, G. (1975) *Biochim. Biophys. Acta* **389**, 51-68
Meissner, G. (1981) *J. Biol. Chem.* **256**, 636-643
Meissner, G., and Young, R. C. (1980) *J. Biol. Chem.* **255**, 6814-6819
Michell, R. H., and Hawthorne, J. N. (1965) *Biochem. Biophys. Res. Commun.* **21**, 333-338
Moore, L., Chen, T., Knapp, H. R., Jr., and Landon, E. J. (1975) *J. Biol. Chem.* **250**, 4562-4568
Nisson, R., Peterson, E., and Dallner, G. (1973) *J. Cell Biol.* **56**, 762-776
Robbins, P. W., Hubbard, S. C., Turco, S. J., and Wirth, D. F. (1977) *Cell* **12**, 893-900
Rouser, G., and Fleischer, S. (1967) *Methods Enzymol.* **10**, 385-406
Sims, P. J., Waggoner, A. S., Wang, C. H., and Hoffman, J. F. (1974) *Biochemistry* **13**, 3315-3330
Snider, M. D., Sultzman, L. A., and Robbins P. W. (1980) *Fed. Proc.* **39**, 1672
Staneloni, R. J., and Leloir, L. F. (1979) *Trends Biochem. Sci.* **4**, 65-67
Tabas, I., Scalesinger S., and Kornfeld, S. (1978) *J. Biol. Chem.* **253**, 716-722
Young, R. C., Allen, R., and Meissner, G. (1981) *Biochim. Biophys. Acta* **640**, 408-418
Zoccoli, M. A., and Karnovsky, M. L. (1980) *J. Biol. Chem.* **255**, 1113-1119