MINIREVIEW

Glucose-6-Phosphatase Structure, Regulation, and Function: An Update[1] (44142)

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Abstract. **Work on the glucose-6-phosphatase system has intensified and diversified extensively in the past 3 years. The gene for the catalytic unit of the liver enzyme has been cloned from three species, and regulation at the level of gene expression is being studied in several laboratories worldwide. More than 20 sites of mutation in the catalytic unit protein have been demonstrated to underlie glycogenesis type 1a. Inhibition of glucose-6-P hydrolysis by several newly identified competitive and time-dependent, irreversible inhibitors has been demonstrated and in several instances the predicted effects on liver glycogen formation and/or breakdown and on blood glucose production have been shown. Refinements in and additions to the presently dominant "substrate transport-catalytic unit" topological model for the glucose-6-phosphatase system have been made. A new model alternative to this, based on the "combined conformational flexibility-substrate transport" concept, has emerged. Experimental evidence for the phosphorylation of glucose in liver by high-K_m,glucose enzyme(s) in addition to glucokinase has continued to emerge, and new *in vitro* evidence supportive of biosynthetic functions of the glucose-6-phosphatase system in this role has appeared. High levels of multifunctional glucose-6-phosphatase have been shown present in pancreatic islet β cells. Glucose-6-P has been established as the likely insulin secretagog in β cells. Interesting differences in the temporal responses of glucose-6-phosphatase in kidney and liver have been demonstrated. An initial attempt is made here to meld the hepatic and pancreatic islet β-cell glucose-6-phosphatase systems, and to a lesser extent the kidney tubular and small intestinal mucosal glucose-6-phosphatase systems into an integrated, coordinated mechanism involved in whole-body glucose homeostasis in health and disease.** [P.S.E.B.M. 1997, Vol 215]

We last reviewed recent developments in the glucose-6-phosphatase area in 1993 (1). Since then, many exciting things have happened in this rapidly evolving field. The reader is directed to several informative reviews of the glucose-6-phosphatase system, its topology, its molecular biology, its metabolic roles, and its regulation, which have appeared since our review in 1993 (1). Gérald van de Werve and his associates (2) authored a brief but comprehensive review (in French) of the system, its components, kinetics under varying conditions, inborn defects, and regulation. Ann Burchell and her colleagues

[1] This manuscript is an update of a previously published minireview entitled, "Recent advances in hepatic glucose 6-phosphatase regulation and function" (Proc Soc Exp Biol Med **203**:274–285, 1993).

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have published several valuable reviews in this time frame, including general considerations of components of the glucose-6-phosphatase system (3, 4), the temporal appearance of the several components of the system in various tissues during human development (5), and the involvement of components of the glucose-6-phosphatase system in hepatic phosphate (6) and glucose (7) transport. Gilles Mithieux (8) has just published a very up-to-date review covering work on the structure, genetics, regulation, and some metabolic roles of glucose-6-phosphatase. An entire issue of the *European Journal of Pediatrics* (9) has been devoted to brief reviews of many aspects of glucose-6-phosphatase based on papers presented at the International Conference on Glycogen Storage Disease Type I held in Fulda, Germany.

The general reader is alerted to the fact that the glucose-6-phosphatase field is not without strong differences of opinion and accompanying controversies (as it should be in science), and that the various reviews, including the present, reflect these differing points of views of the authors.

As earlier (1), we have by choice limited ourselves here generally to a review of selected works, those from the literature appearing between 1993 and the present, with a special focus on our own work and a synthesis of our ideas regarding the topological organization, regulation, and especially, the physiologic functions of the complex glucose-6-phosphatase system. Most of the studies worldwide with this enzyme focus upon that in isolated liver "microsomes." This review reflects that fact. In addition, it contains important new information relating to the multifunctional enzyme in β cells of the pancreas, and to a lesser extent kidney, and small intestine. We conclude with an attempt at integration, in which we combine our previously articulated (1, 10) "tuning/returning" hypothesis involving both hydrolytic and biosynthetic activities of glucose-6-phosphatase of the liver, with the multifunctional enzyme now known to be present in pancreatic islet cells, kidney tubules, and mucosa of the small intestine. Activities of the enzyme in all these tissues are proposed to be involved in a coordinated way, along with glucokinase in liver and pancreatic β cells, for the control of blood glucose levels of the body normally, and in the readjustment of these levels progressively as diabetes develops and in other instances characterized by moderate elevations in blood glucose levels, for instance, in the aged and in birds, as well.

Topological Considerations

Currently, there are two proposed concepts of structure-function relationship for glucose-6-phosphatase, the "substrate transport-catalytic unit" concept and the "combined conformational flexibility-substrate transport" concept. Both concepts have received support over the years, but neither fully explains all of the experimental data available.

The Substrate Transport-Catalytic Unit Model. The involvement of glucose-6-P transport in glucose-6-phosphatase function was first proposed in 1975 by Arion and co-workers (11), who later developed the substrate transport-catalytic unit concept (12) based on a variety of kinetic data (11–13). A current depiction of the substrate transport-catalytic unit concept is shown in Figure 1A. This model incorporates the many relevant hydrolytic and synthetic activities of glucose-6-phosphatase in liver endoplasmic reticulum, and proposes that glucose-6-phosphatase activities are achieved through a multicomponent system. Glucose-6-phosphatase is capable of catalyzing the hydrolysis of glucose-6-P (Reaction 1 below) and inorganic pyrophosphate (PP_i) (Reaction 4) as well as the synthesis of glucose-6-P *via* potent phosphotransferase activity (Reactions 2 and 3):

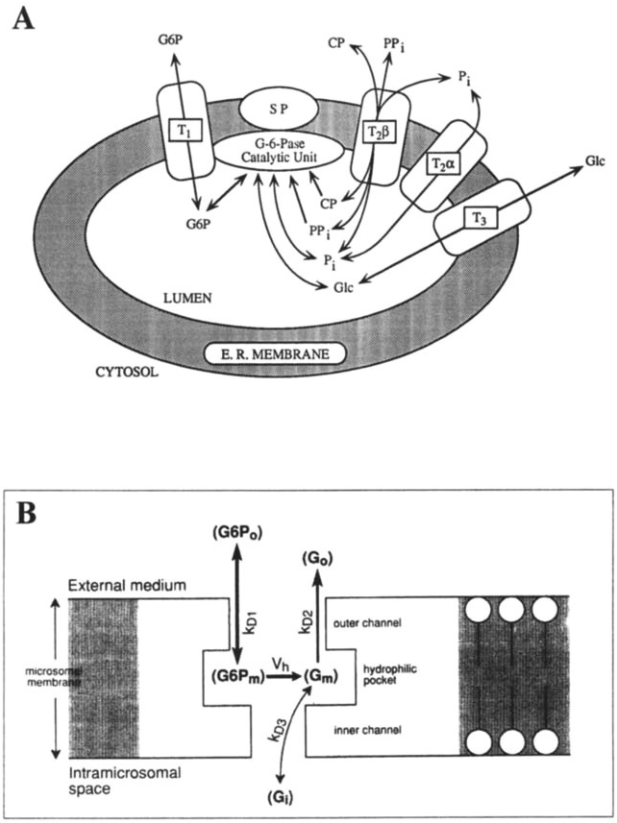

Figure 1. Postulated structure-function relationships of glucose-6-phosphatase. The substrate translocase-catalytic unit model (A) by Foster *et al.* (14) depicts a cross section of the endoplasmic reticulum (E. R. MEMBRANE): SP, stabilizing protein; T_1, $T_2\alpha$, $T_2\beta$, and T_3, substrate/product transporters with the indicated specificity; Catalytic unit, glucose-6-phosphatase (EC 3.1.3.9) enzyme. Two forms of T_2 with differing specificity, termed $T_2\alpha$ and $T_2\beta$, have been proposed (15). Panel A is from Foster *et al.* (14) by permission of Elsevier Science B.V. A modified version of the combined conformational flexibility-substrate transport model (B) proposed by Berteloot *et al.* (16) depicts glucose-6-phosphatase as a transmembrane protein with the catalytic site lying in a hydrophilic pocket deep inside of the protein where G6P hydrolysis occurs at a rate V_h. Exchanges between the extra- and intravesicular spaces are made possible through outer and inner channels with different intrinsic permeabilities to glucose (k_{D2},k_{D3}) and G6P (k_{D1}). G6P and G, glucose-6-P and glucose concentrations; indices o, m, and i, the incubation medium, the hydrophilic pocket, and the intramicrosomal space, respectively. Panel B is from Berteloot *et al.* (16), by permission of the American Society for Biological Chemistry and Molecular Biology. This figure is reproduced from Foster *et al.* (17), by permission of Elsevier Science B.V.

$$\text{Glucose-6-P} + H_2O \rightarrow \text{glucose} + P_i \quad [1]$$

$$\text{Carbamyl-P} + \text{glucose} \rightarrow \text{glucose-6-P} + NH_3 + CO_2 \quad [2]$$

$$PP_i + \text{glucose} \rightarrow \text{glucose-6-P} + P_i \quad [3]$$

$$PP_i + H_2O \rightarrow 2\ P_i \quad [4]$$

According to the substrate transport-catalytic unit concept, these reactions are achieved by a system consisting of a fairly nonspecific phosphohydrolase/phosphotransferase catalytic unit with its active site located on the lumenal side of the endoplasmic reticulum and at least four transmembrane spanning translocases (Fig. 1A). The gene coding for the catalytic unit of this system has been isolated for the liver enzyme in the mouse (18), rat (19), and human (20). Molecular biological aspects of the catalytic unit will be discussed in more detail in a subsequent section of this review. Associated translocases confer specificity to this system by allowing selective substrates/products access to or egress from the sequestered catalytic unit.

Translocase T_1, the putative glucose-6-P–specific transporter, has not been identified. Ann Burchell, Angelo Benedetti, and co-workers have provided evidence that glucose-6-P is translocated into the lumen of liver microsomal vesicles (21). An osmotically induced decrease in light-scattering intensity in the presence of glucose-6-P but not mannose-6-P revealed selective permeability of microsomal vesicles to glucose-6-P. Sarcoplasmic reticulum of skeletal muscle, in contrast to liver microsomes, did not show selective permeability to glucose-6-P because mannose-6-P was also permeable to these vesicles (22).

Several compounds have been postulated to inhibit glucose-6-phosphatase by interacting with putative translocase T_1 (23). These compounds inhibit glucose-6-P hydrolysis in intact microsomes, but inhibition is not observed when microsomes are disrupted by exposure to detergent or by mechanical means. Recently, two additional compounds, 3-mercaptopicolinate (3-MP) (14) and *N*-bromoacetylethanolamine phosphate (BAEP) (17), have been added to this list. These two new compounds inhibit glucose-6-P hydrolysis in intact microsomes in a time- and concentration-dependent manner. Disruption of the microsomal integrity by detergent treatment prior to incubation with either inhibitor or subsequent to preliminary incubation with either inhibitor but prior to assay for activity abolishes the time-dependent inhibition (14, 17). These irreversible, time- and concentration-dependent inhibitory actions of 3-MP and BAEP are manifest at a site where the intact membrane-bound enzyme first makes contact with the substrate glucose-6-P. The irreversible, time- and concentration-dependent inhibition by BAEP, in particular, along with our ability to synthesize radiolabeled BAEP, strongly suggests the potential utility of this compound as an affinity label for the identification of putative auxiliary component, T_1, of the glucose-6-phosphatase system (17).

One form of translocase T_2, $T_2\beta$, with a molecular mass of 37 kDa, has been identified and purified with the use of antibodies raised against the rat mitochondrial phosphate/hydroxyl ion antiport protein (24). Studies with human hepatic microsomes in which $T_2\beta$ has been shown immunologically to be absent provided evidence that the hepatic microsomal glucose-6-phosphatase system must contain an additional T_2 component, $T_2\alpha$ (15). In these studies, P_i the product of glucose-6-P hydrolysis, did not accumulate within the lumen of the microsomes although these microsomes were devoid of the immunologically reactive form of T_2, $T_2\beta$. These results suggested the existence of an additional high-K_m translocase which would transport P_i out of the microsomal lumen when P_i concentration becomes high and $T_2\beta$ is absent (Fig. 1A). This additional translocase was termed $T_2\alpha$ (15). The presence of Ehrlich-ascites tumors *in situ* has been shown to affect the hepatic glucose-6-phosphatase system in mice (25, 26). The most notable effect was a 2.5-fold increase in the quantity of the $T_2\beta$ protein (25). In addition, the markedly lowered ability of exogenously added P_i to inhibit glucose-6-P hydrolysis in intact microsomes derived from tumor-bearing mice suggested the appearance of a novel form of the $T_2\beta$ translocase in these tumor-stressed mice (26).

Translocase T_3, a 52-kDa glucose-transport protein, has been isolated by Burchell and co-workers from rat liver microsomes (27). A cDNA clone was isolated using antibodies raised against the T_3 protein (28). This translocase has been termed GLUT 7 because of its sequence similarities to GLUTs 1–6 (7, 28). The deduced amino acid sequence of GLUT 7 shows 68% homology with that of rat liver GLUT 2 (28). Microsomes isolated from COS 7 cells transfected with the GLUT 7 clone showed an increase in microsomal glucose-transport capacity, demonstrating that the GLUT 7 clone encodes a functional endoplasmic reticulum glucose-transport protein (28).

The Combined Conformational Flexibility-Substrate Transport Model. A model alternative to the substrate translocase-catalytic unit concept was first introduced by Schulze and co-workers in 1986 (29). This new model was termed the "combined conformational flexibility-substrate transport" model and depicts glucose-6-phosphatase as a multifunctional enzyme embedded deep within the endoplasmic reticulum membrane possessing both catalytic and substrate/product transport activities. This concept has received support from van de Werve and co-workers who have demonstrated a presteady-state "burst" in the rate of glucose-6-P hydrolysis in untreated microsomes using a fast-sampling, rapid-filtration technique (30). The rate of hydrolysis in this burst phase matched the steady-state rate of glucose-6-P hydrolysis in detergent-treated vesicles (30). This observation is inconsistent with a rate-limiting transport step as proposed by the substrate-translocase concept. Gérald van de Werve and co-workers proposed that a hysteretic transition best explained their results and suggested that there was a tight coupling between glucose-6-P transport and hydrolysis (30). This view was supported by the more recent finding of van de

Werve and co-workers that no accumulation of radiolabel (^{14}C-glucose-P or ^{14}C-glucose) was observed in microsomes derived from a glycogen storage disease type 1a patient (31).

Mithieux and co-workers have recently confirmed this burst phenomenon and, additionally, have demonstrated that glucose-6-phosphatase is able to hydrolyze both glucose-6-P and mannose-6-P at very similar rates during the pre-steady-state/hysteretic transition period (32). Only glucose-6-P is hydrolyzed at a significant rate during the steady-state period, but after detergent-treatment of the microsomal membrane, glucose-6-P and mannose-6-P are again hydrolyzed at similar rates (32). However, Mithieux and co-workers have reported a limited specific kinetic behavior towards glucose-6-P after detergent-treatment of the membrane (33).

In 1995, van de Werve and co-workers (16) proposed a modified version of the combined flexibility-substrate transport model (Fig. 1B) based on their observation that the rapid phase of tracer (^{14}C-glucose) accumulation into microsomal vesicles and the burst phase in glucose-6-P hydrolysis appear synchronously (34), and kinetic evidence of a membrane-exchangeable glucose pool (16). Gérald van de Werve and co-workers proposed a model (Fig. 1B) in which the catalytic site of glucose-6-P hydrolysis lies deep within a hydrophilic pocket of an intrinsic membrane protein which is connected to the extra- and intravesicular spaces through channels with different intrinsic permeabilities to glucose (16). In regard to glucose permeability, they have demonstrated that glucose efflux from microsomal vesicles occurs independently of hydrolysis (34) but were unable to show uptake of free radiolabeled glucose into microsomal vesicles (35). The upper channel in this new model is the proposed site of action of histone 2A (16), which stimulates glucose-6-phosphatase activity to the same extent as detergent treatment (36). Unlike detergents however, histone 2A does not seem to permeabilize the microsomal membrane because the accumulation of ^{14}C-glucose from ^{14}C-glucose-6-P into microsomes is still observed and actually increases in the presence of histone 2A (36).

Some General Considerations. Although neither the current substrate transport-catalytic unit model nor the modified combined conformational flexibility-substrate transport model adequately explains all of the experimental data acquired at the present time, the substrate transport-catalytic unit model has been and will likely remain the dominant model for glucose-6-phosphatase because it attempts to incorporate all of the various hydrolytic and synthetic activities of glucose-6-phosphatase whereas the combined conformational flexibility-substrate transport model does not. The existence of a multicomponent system is strongly supported by the work of Chou and co-workers (37), which demonstrates that the gene for the catalytic subunit of glucose-6-phosphatase is normal in patients diagnosed with glycogen storage diseases type 1b and 1c. In addition, Chou and co-workers have demonstrated that there is no glucose-6-P uptake (i.e., glucose accumulation) in microsomes derived from glucose-6-phosphatase knock-out mice, which mimic the pathophysiology of human GSD-1a patients (38). This supports the previous work of van de Werve *et al.* suggesting a tight coupling between glucose-6-P transport and hydrolysis (30). Chou and co-workers, however, have also demonstrated differential inhibitory sensitivity of glucose-6-P transport and hydrolysis by the competitive inhibitor vanadate in liver and kidney microsomes (38). This latter finding suggests the involvement of two separate but tightly coupled proteins in the transport and hydrolysis of glucose-6-P.

A final model representing the true topological makeup of glucose-6-phosphatase and its associated proteins is likely to contain aspects of both the substrate transport-catalytic unit concept and the combined conformational flexibility-substrate transport concept.

Molecular Biology of the Catalytic Unit

Cloning of the Catalytic Unit. Despite efforts by veteran researchers in the glucose-6-phosphatase field, membrane-bound glucose-6-phosphatase has eluded molecular characterization for many years. Recently, however, Janice Chou and co-workers were able to isolate cDNA encoding murine glucose-6-phosphatase (18) by taking advantage of an albino deletion mutant mouse which expressed markedly reduced levels of glucose-6-phosphatase activity. The isolation of glucose-6-phosphatase cDNA was achieved by screening a mouse liver cDNA library differentially with mRNA populations representing the normal and the albino deletion mutant mouse (18). Currently, the gene coding for the catalytic unit of glucose-6-phosphatase (EC 3.1.3.9) has been isolated for the liver enzyme of the mouse (18), rat (19), and human (20). The human glucose-6-phosphatase catalytic unit gene is a single copy gene and is located on chromosome 17 (39). The human gene spans 12.5 kb and consists of 5 exons: I (309 bp), II (110 bp), III (106 bp), IV (116 bp), and V (2451 bp including a coding region of 509 bp) (Fig. 2). The catalytic unit protein consists of 357 amino acids with an apparent molecular weight of 35 kDa (20). The deduced human catalytic protein is extremely hydrophobic and contains six putative membrane-spanning segments as well as the endoplasmic reticulum protein retention signal, KK, located at residues 354 and 355 (20) (Fig. 3). In addition, the deduced protein has three potential Asn-linked glycosylation sites (20). Conservation of amino acid sequence is very high across all three species (92%–

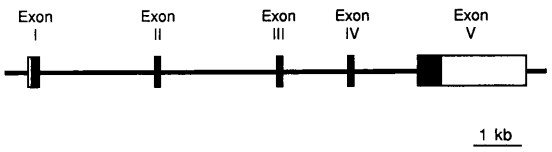

Figure 2. The structural organization for the transcriptional unit of the human glucose-6-phosphatase catalytic unit. Exon coding regions are indicated by filled boxes and untranslated regions by open boxes.

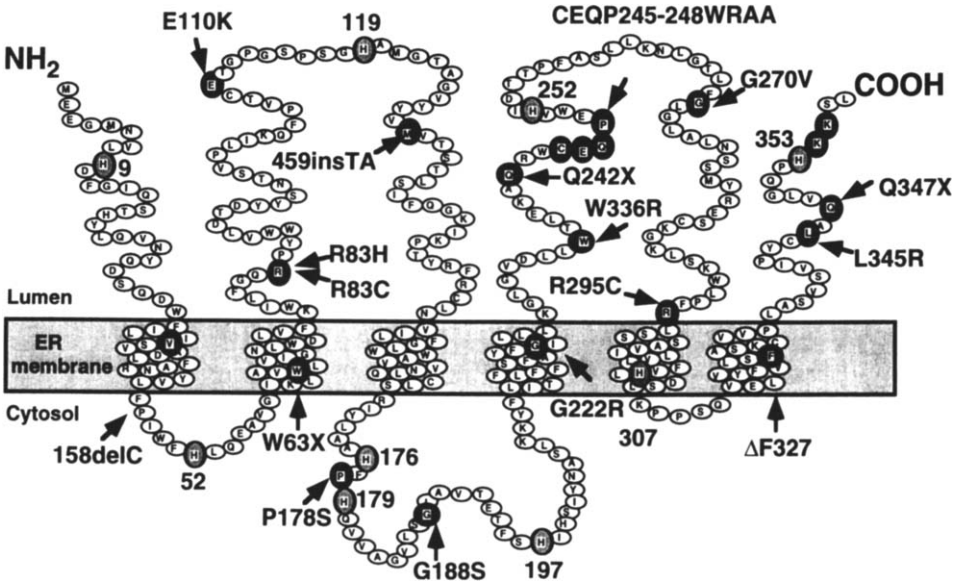

Figure 3. The predicted secondary structure of the human glucose-6-phosphatase catalytic unit and the location of several mutations identified in GSD type 1a patients. Transmembrane-spanning domains were identified by Chou and co-workers using the method of Klein *et al.* (40) and the PC/Gene computer program. Mutations are highlighted and denoted by arrows. His residues are highlighted and numbered. The figure was provided through the courtesy of Dr. Janice Chou and is modified from Lei *et al.* (37) and Lei *et al.* (41).

95% homology) studied thus far (42). The six putative transmembrane-spanning domains, the three potential glycosylation sites and the ER retention signal are all conserved across the deduced amino acid sequence for the human, mouse, and rat catalytic unit proteins (42). In addition, nine conserved His residues exist in the human glucose-6-phosphatase protein (41). Mutational analysis of these His residues (41) suggested that His-119 is the phosphate acceptor in glucose-6-phosphatase catalysis (Fig. 3).

Inborn Errors in the Catalytic Unit Identified. The absence of a functional catalytic unit protein is the cause of type 1a glycogen storage disease (GSD), an autosomal recessive disorder with an incidence of 1 in 100,000–300,000. The disease presents with clinical manifestations of severe hypoglycemia, hepatomegaly, growth retardation, lactic acidemia, hyperlipidemia, and hyperuricemia (43, 44). Numerous genetic mutations have been identified in patients diagnosed with type 1 glycogen storage disease. Chou and co-workers have characterized mutations in the glucose-6-phosphatase gene of 70 unrelated type 1a GSD patients of different ethnic backgrounds (45). In addition they have characterized the glucose-6-phosphatase gene of one GSD type 1c, a single type 1aSP, and three type 1b patients (37). The gene of GSD type 1b and 1c patients was found to be normal (37). However, a mutation in exon 2 of the glucose-6-phosphatase gene that converts an Arg at codon 83 to a Cys (R83C) was identified in both alleles of the type 1aSP patient (37). These studies indicate that at least two loci are involved in the hydrolysis of glucose-6-P, and that GSD type 1aSP, like type 1a, is caused by mutations that inactivate the glucose-6-phosphatase catalytic unit (37). Mutations in the glucose-6-phosphatase gene of 24 French (46), 8 Chinese (47), 7 Israeli (48), and 11 Japanese (49) patients have also been characterized recently. A listing of all mutations in glucose-6-phosphatase gene alleles of GSD type 1a patients reported to date is shown in Table I. Prevalent mutations found among various ethnic groups are shown in Table II. The prevalent mutation found among Japanese patients is of particular interest because it is a novel splicing mutation in the glucose-6-phosphatase gene which results in a glucose-6-phosphatase protein that is 146 amino acids shorter than the normal gene product (49). A single nucleotide substitution (G to T) at position 727 of the gene is thought to be the cause of the mutation (49). Although the splice site in intron 4 and exon 5 has a normal

Table I. Mutations in the Glucose-6-Phosphatase Gene of GSD Type Ia Patients

Mutation	Reference
R83C	20, 45, 46, 48
Q347X	45, 46
130X (459 ins TA)	20, 45
R83H	45
R83I	47
V166G	48
ΔF327	37, 45
R295C	20, 45
G222R	37, 45
D38V	46
W77R	46
E110K	46
A124T	46
G184E	46
G188R	46
L211P	46
35X (158 del C)	45
Q242X	45
W63X	45
G188S	45
P178S	45
W236R	45
CEQP245-248WRAA	45
G270V	45
L345R	45
254X	46
G727T (splice site)	49
A313G (splice site)	46

Table II. Prevalent Mutations in the Glucose-6-Phosphatase Gene among Various Ethnic/Racial Groups

Ethnic/racial group	Prevalent mutation(s)
Caucasian	R83C
	Q347X
French	Q347X
	R83C
Hispanic	130X
	R83C
Israeli	
Jewish	R83C
Arab	V166G (1 case reported)
Chinese	R83H
	R83I
Japanese	G727A (splice site)

consensus sequence after this nucleotide substitution, a normal splice does not occur (49).

The various mutations above nicely describe the underlying cause of type 1a GSD. Patients diagnosed with type 1b or type 1c GSD, however, do not have mutations present in their gene for the glucose-6-phosphatase catalytic unit (37). This strongly indicates that there are additional factors involved in the process by which glucose-6-P is hydrolyzed in the endoplasmic reticulum. Recently, Burchell and co-workers have reported a GSD patient thought to have multiple transport protein defects and have classified this GSD patient as type $1b/1c_{\beta}$ (50). It will be interesting to see if this patient, like type 1b and 1c patients, has a normal glucose-6-phosphatase catalytic unit gene.

Regulation of Catalytic Unit Gene Expression. With the isolation of the gene and the availability of cDNA clones (18–20, 42) for the catalytic unit of glucose-6-phosphatase it is now possible to study the expression of the gene under various nutritional and hormonal states. Pilkis, Lange, and co-workers were the first to describe the regulation of glucose-6-phosphatase gene expression in terms of relative mRNA levels in FAO hepatoma cells (42). In these studies FAO cells were incubated with insulin, dexamethasone and the cAMP analog CPT-cAMP for 24 hr, individually or in combination. Dexamethasone increased glucose-6-phosphatase mRNA over basal levels and insulin greatly decreased mRNA levels. In cells incubated with both dexamethasone and insulin, insulin completely blocked the increase seen with dexamethasone alone. CPT-cAMP had little effect on glucose-6-phosphatase mRNA levels when added alone, but inhibited insulin's suppression of gene expression. It was also noted in these studies that the expression of glucose-6-phosphatase mRNA (i.e., relative mRNA levels) in FAO cells was much greater in the presence of glucose (25 mM) than in its absence (42).

Rossetti and co-workers have recently reported studies utilizing 90% partially pancreatectomized diabetic rats which indicate that *in vivo* gene expression of glucose-6-phosphatase in the liver is regulated by glucose independently from insulin (51). The relative abundance of glucose-6-phosphatase mRNA increases in the diabetic state (19, 51, 52) and is normalized when plasma glucose concentration is decreased in the absence of insulin (51).

Increases in glucose-6-phosphatase gene expression have been reported in rats after the induction of hemorrhagic shock (53) and also in the regenerating rat liver after partial hepatectomy (54).

Burchell and co-workers have recently cloned the 5′ region of the human glucose-6-phosphatase gene (55) and have reported transcriptional regulation by dibutyryl cAMP, insulin, and dexamethasone. The results of these studies (55) closely resemble those found in the studies of Pilkis, Lange, and co-workers (42) in which the regulation of rat glucose-6-phosphatase gene expression was examined (see above). The 5′-flanking region of the rat glucose-6-phosphatase gene was recently isolated by Lange and co-workers (19). Several *potential* promoter-related sequences have been identified in both the human (55) and rat (19) glucose-6-phosphatase gene 5′-flanking region, including TATAAA- and CCAAT-boxes; HNF and AP-1 transcription factor binding sites; and three response elements (CRE, GRE, and IRE).

Acute Regulation of the Glucose-6-Phosphatase System by Inhibitors and Activators and Demonstration of Metabolic Consequences

Evidence continues to accumulate indicating that acute regulatory mechanisms other than substrate concentration alone (56) exist for glucose-6-phosphatase. A recent study by Mithieux and co-workers showed that glucose-6-phosphatase was inhibited in rats a short time after refeeding (57). The inhibition involved a decrease in the V_{max} and was observed in homogenates from livers freeze-clamped *in situ* but not in microsomes isolated from those homogenates. This suggests that the inhibitor was either highly labile or that inhibition was dependent on a metabolite present in the homogenates that was lost during the microsome isolation process (57). We have previously demonstrated the inhibition of glucose-6-phosphatase by an unidentified proline metabolite in isolated perfused rat liver (58), which suggests that the hypothesis of a metabolite-induced inhibitory mechanism in the studies of Mithieux and co-workers (57) is a very plausible one. Another unidentified inhibitor of glucose-6-phosphatase which is associated with glycogen has been reported by Barret and co-workers (59). This endogenous glycogen-associated compound inhibited glucose-6-phosphatase activity in intact and detergent-treated microsomes, and could be dissociated from glycogen by ion-exchange and ultrafiltration (59). The hypoglycemic effect of *Coccinia indica* leaves and the vegetable *Momordica charantia* has been attributed to a decrease in glucose-6-phosphatase activity in a study by Rahman and co-workers (60). A 32% decrease in glucose-6-phosphatase activity was seen 90 min after oral administration of *Coccinia indica* leaf extracts to streptozotocin-diabetic rats. A discrete inhibitory

factor has not been identified in this study, but the mechanism would seem to involve an acute regulatory step.

Two reports of glucose-6-phosphatase activators have recently appeared in the literature. An unidentified heat sensitive activator of glucose-6-phosphatase found in a protein-free filtrate has been reported by Pilkis, Lange, and co-workers (53). Protein-free filtrates derived from the supernatant fraction of microsomal preparations from the liver of hemorrhagic shock rats, but not control rats, increased glucose-6-phosphatase activity (53). However, when control and hemorrhagic shock protein-free filtrates were heated, they both increased glucose-6-phosphatase activity to the same extent (53). Activation of glucose-6-phosphatase has also been demonstrated in hepatocytes in the presence of protein phosphatase inhibitors (microcystin-LR, okadaic acid, calyculin A, and microcystin-RR) by Lavoinne, Claeyssen, and co-workers (61). Promycin, an inhibitor of protein synthesis, totally suppressed the activating effect. However, a later study by the same group (62) revealed that these protein phosphatase inhibitors had a general inhibitory effect on protein synthesis in hepatocytes which preceded the activation of glucose-6-phosphatase. The link between the inhibition of protein synthesis and glucose-6-phosphatase activation remains to be established (62).

The inhibition of glucose-6-phosphatase *in vitro* by fatty acyl-CoA esters and unsaturated fatty acids has been examined in greater detail over the past 3 years. In 1993, Mithieux and co-workers reported that the polyunsaturated fatty acid, arachidonic acid, inhibited glucose-6-phosphatase activity in a dose-dependent manner between 10 and 100 μM (63). IC_{50} values of 27.5 and 24 μM for untreated and detergent-treated microsomes, respectively, were determined and seem consistent with K_i values of 2.5–17 μM (depending on the conditions and activity studied) determined in the senior author's laboratory (1). An intriguing finding in the study by Mithieux and co-workers was that glucose-6-phosphatase of untreated microsomes from diabetic rats was less inhibitable by arachidonic acid than that of normal rats (63). In 1995, Burchell, Benedetti and co-workers reported studies that examined the inhibitory effects of the fatty acyl-CoA esters on rat liver microsomal glucose-6-phosphatase (64). They demonstrated that palmitoyl-CoA decreased the microsomal permeability to glucose-6-P, but not to glucose or phosphate, indicating that the putative transport protein, T_1, was the site of palmitoyl-CoA binding and inhibition of glucose-6-phosphatase (64). Later, in 1996, Mithieux and Zitoun studied the effect of short-, medium-, and long-chain fatty acyl-CoA esters on glucose-6-phosphatase activity in untreated and detergent treated liver microsomes (65). Short-chain fatty acyl-CoA esters (less than or equal to nine carbons) did not inhibit glucose-6-phosphatase. Medium-chain fatty acyl-CoA esters (10–14 carbons) inhibited glucose-6-phosphatase of untreated microsomes in a dose-dependent manner in a range of 1–20 μM and long-chain fatty acyl-CoA esters (equal to or greater than 16 carbons) inhibited within a range of 1–2 μM. However, this inhibitory effect was partially or totally abolished, or resulted in an activation of glucose-6-phosphatase when higher concentrations of long-chain fatty acyl-CoA esters were utilized (65). This observation is consistent with that seen earlier in the senior author's laboratory in studies with palmitoyl-CoA (66). The activation of glucose-6-phosphatase with higher concentrations of long-chain fatty acyl-CoA esters is due to detergent-like effects of these compounds at higher concentration (65).

Recently, we have examined the inhibitory effects of *N*-bromoacetylethanolamine phosphate (BAEP) on the glucose-6-phosphatase system (17). This compound binds irreversibly to the putative T_1 translocase. The inhibitory effects of BAEP on glucose-6-P phosphohydrolase activity were much like those seen in earlier studies with 3-mercaptopicolinate (3-MP) (14). In liver perfusion studies (67), we demonstrated that the inhibition of glucose-6-P phosphohydrolase by 3-MP in isolated perfused livers from 48-hr fasted rats was metabolically directive by enhancing glycogenesis from glucose, dihydroxyacetone, and fructose. In later studies (14), we demonstrated the effectiveness of 3-MP as a time-dependent inhibitor of glucose-6-phosphatase, *in situ,* by measuring glycogenolysis in livers from fed rats (Fig. 4). Together, these two studies nicely demonstrate the metabolic consequences of the inhibition of glucose-6-P hydrolysis *in situ.*

Recent Evidence Supportive of the Participation of Phosphotransferase Activity of the Glucose-6-Phosphatase System in Hepatic Glucose Phosphorylation

The multiplicity of reactions, both hydrolytic and biosynthetic, catalyzed by the glucose-6-phosphatase system have been described in detail above and earlier (1, 10). In our preceding *P.S.E.B.M.* review (1) and elsewhere (e.g., Refs. 10, 68–70) we have described evidence from the literature supportive of roles for biosynthetic activity of the glucose-6-phosphatase system (e.g., carbamyl-P:glucose phosphotransferase) in hepatic glucose phosphorylation. Evidence continues to accumulate indicating generally the presence in liver of a high-K_m,glucose enzyme activity or activities capable of glucose phosphorylation independently of glucokinase (10, 68). New data have been published in the past 3 years that we believe are strongly supportive specifically of biosynthetic activity of the glucose-6-phosphatase system in this role. We would point out, however, that our conclusions, usually based on *in vitro* work extrapolated to the *in vivo* condition, are still considered controversial by some authorities.

Below, we summarize these newly published findings. In the last major section of this review, we propose a coordinated, integrated system involved prominently in mammalian blood glucose homeostasis which incorporates hydrolytic and biosynthetic activities of the glucose-6-phosphatase system of liver hepatocytes and pancreatic islet β cells along with glucokinase. Roles for activities of mul-

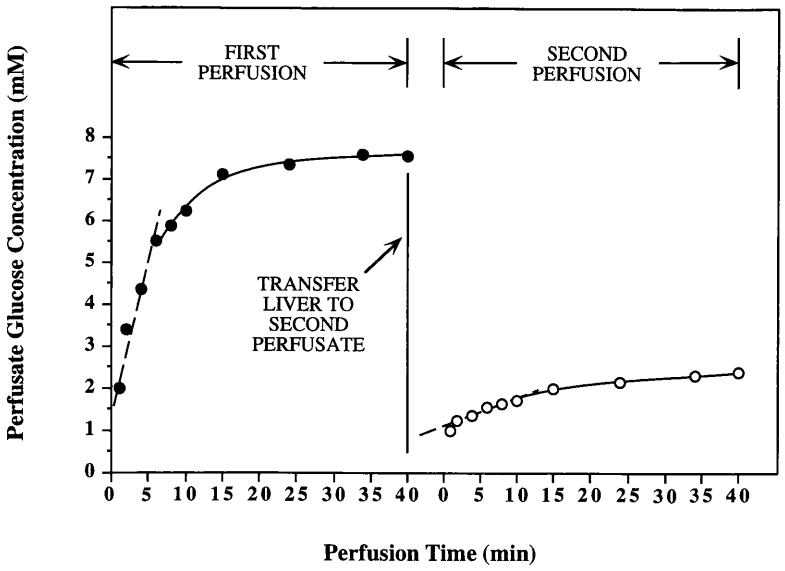

Figure 4. Time-dependent inhibition by 3-MP of glycogenolysis in an isolated, perfused liver from a fed rat. The liver was connected to the first perfusion system supplemented with 3-MP to 2.0 m*M*, and perfusions were carried out for 40 min (FIRST PERFUSION). This allowed sufficient time for time-dependent inhibition by 3-MP of glucose-6-phosphatase to develop. The liver was then removed from the first system and attached to a second system of identical initial composition but devoid of endogenous glucose, and perfusion was continued for a second 40 min (SECOND PERFUSION). Perfusate samples were removed at the times indicated, and glucose concentration was determined. Hepatic glycogen was measured in biopsy samples taken just before initiation of the first perfusion and immediately following the first and second perfusions. The 3-MP was replaced by an equal volume of saline in control studies (not shown). Dashed lines represent the initial linear portion of the glucose-release phase upon which glycogenolytic rates are based. The initial glycogenolytic rates for the control in the first and second perfusion period were 3.48 ± 0.23 and 1.86 ± 0.11, respectively. The initial glycogenolytic rates in the first and second perfusion period in the presence of 3-MP were 3.45 ± 0.53 and 0.94 ± 0.16, respectively. Perfusate glucose concentrations achieved in control studies after 40 min of perfusion were 7.91 ± 0.20 m*M* in the first perfusion period and 5.07 ± 0.21 m*M* in the second perfusion period. This figure is reproduced from Foster *et al.* (14), by permission of Elsevier Science B.V.

tifunctional glucose-6-phosphatase of kidney tubules and small intestinal mucosa are also proposed.

Recent Experimental Evidence in Support of High-K_m,glucose Enzyme(s) in Addition to Glucokinase in Hepatic Glucose Phosphorylation. In his 1970 textbook, Albert Lehninger (71) recognized the need for the function of a high-K_m,glucose hepatic enzyme which should be especially active to phosphorylate, and thus help to control, high levels of blood glucose as encountered in untreated diabetes. He erroneously attributed this function to glucokinase. Hepatic glucokinase gene expression requires insulin as an inducer, and hence that activity would be low or absent when seemingly most needed. We have proposed (10, 68–70) that biosynthetic activity of the glucose-6-phosphatase system, which is favored in uncontrolled diabetes both through increased active enzyme protein (72) and by elevated levels of glucose as substrate (its K_m,glucose is ~40 m*M*), could play this role. In our earlier review (1), we summarized evidence supportive of hepatic glucose phosphorylation by an enzyme or enzymes in addition to glucokinase. Included prominently there was the demonstration of a high-K_m,glucose value for hepatic glucose phosphorylation which was >> that for glucokinase (68, 73) and the demonstration that isolated perfused livers derived from experimentally diabetic rats show net glucose uptake at glucose concentrations at and above 4.5 m*M* when 3-mercaptopicolinate was added to block endogenous gluconeogenesis at the phosphoenolpyruvate carboxykinase level (74).

Very recent evidence supports both of these findings. Wals and Katz (75) studied net glucose uptake, glycogenesis, and lactate production as a function of glucose concentration in isolated rat hepatocytes and showed these processes were not saturated by glucose even at the 80 m*M* level. More recently, these same workers (76) measured glucose phosphorylation and cycling between glucose and glucose-6-P in hepatocytes derived from livers of fasted rats. They concluded that their "results indicate that the rate of phosphorylation [of glucose] by hepatocytes of fasted rats is high and difficult to account for by the conventional kinetic parameters of isolated glucokinase" (76). Our own recent studies (14, 67) show that in addition to effects on phosphoenolpyruvate carboxykinase, 3-mercaptopicolinate inhibits glucose-6-P phosphohydrolase but not carbamyl-P:glucose phosphotransferase activity of the glucose-6-phosphatase system, thus allowing glucose phosphorylation with minimal glucose-6-P rehydrolysis, as seen in our earlier perfusion studies (74) with livers from diabetic rats.

Bode, Foster, and Nordlie (77) studied the reciprocity between ureagenesis and glycogenesis from glucose in isolated perfused livers derived from 48-hr fasted rats and manipulated experimental variables including ammonium ion concentration, presence or absence of norvaline or ethoxyzolamide, and glucose concentration. Under conditions favoring glycogenesis from glucose (ammonium ion and the ornithine transcarbamylase inhibitor norvaline present), glycogenesis was much higher with 34 m*M* than with 9 m*M* glucose. A "K_m,glucose" value >30 m*M* was calculated for the glycogenic process, considerably larger than can be attributed exclusively to glucokinase under any defined conditions (77).

Our earlier demonstration of net glucose phosphoryla-

tion in isolated, perfused livers from experimentally diabetic rats (74), cited above, is supported by a recent, independent experimental approach by Henly, Phillips, and Berry (78). They compared rates of glucose/glucose-6-P cycling in hepatocytes derived from normal and streptozotocin-diabetic rats, using 80 m*M* glucose. In the diabetic compared with normal control preparation, the rate of glucose/glucose-6-P cycling was double the normal value, and glucose phosphorylation was lowered only 30% (78), even though 90% of hepatic glucokinase is lost in the diabetic (68). Clearly, in hepatocytes derived from the diabetic rat's liver the capacity for glucose phosphorylation remains strong, even with the near-total disappearance of glucokinase. In addition, studies with transgenic mice (79) in which the liver but not the pancreatic β-cell glucokinase gene has been knocked out demonstrate that glucokinase is not essential for the phosphorylation of glucose in the liver because these mice have a normal life span.

A novel, newly demonstrated phenomenon bestowing increased glucose-concentration sensitivity upon glucose phosphorylation in hepatocytes comes from the work of Agius (80) and Agius and Peak (81). They demonstrated that with isolated hepatocytes precultured with 5 m*M* glucose, almost all glucokinase is bound and inactive. Exposure of the cells to higher concentrations of glucose triggers the release and concomitant activation of this originally bound glucokinase, thus bestowing doubly a glucose-concentration–dependent sensitivity to glucokinase. The potentiation by glucose in this way of cellular processes such as glycogenesis from glucose is characterized by an A_{50} value (that concentration of glucose giving half-maximal activation) of 15 m*M*. We would point out that this value is considerably smaller than the K_m,glucose shown for glucose phosphorylation in hepatocytes (68, 70, 75, 76) and for net glucose uptake (82) and glycogenesis from glucose (83) in isolated perfused livers derived from 48-hr fasted rats. The existence of a ''regulatory protein'' specific for glucokinase (84) provides another mechanism whereby apparent K_m, glucose for hepatic glucose phosphorylation may be increased because this inhibition is competitive versus glucose. We would point out, however, that at the same time the apparent K_m,glucose is being increased through interaction of glucokinase with this regulatory factor, activity of the enzyme at any finite unit concentration of glucose is decreased by inhibition.

Additional Support for Hepatic Glucose Phosphorylation Specifically by Biosynthetic Activity of the Glucose-6-phosphatase System. Recent and ongoing studies supportive of hepatic glucose phosphorylation by carbamyl-P:glucose phosphotransferase activity of the glucose-6-phosphatase system were described in our *P.S.E.B.M.* review (1). In an early, critical study, Lueck and Nordlie (85) showed directly the coupling of carbamyl-P synthesis by mitochondrial carbamyl-P synthase I with glucose phosphorylation by carbamyl-P:glucose phosphotransferase activity of the microsomal glucose-6-phosphatase system. A reconstituted system was used which included isolated mitochondria and microsomes (fragments of the endoplasmic reticulum) in direct proportion to their content in liver cells. The credibility of biosynthetic activity of the glucose-6-phosphatase system as a participant in hepatic glucose phosphorylation was thus established; the question of whether, and to what extent, this system operates under physiologic or pathologic conditions remained, however (85). Recent evidence, following, bears positively upon this question.

The demonstration of translocase protein(s) specifically for transport of carbamyl-P and PP_i and of glucose into the lumen of the endoplasmic reticulum, and of glucose-6-P out (1, 3–7; see also an earlier discussion, above), raises the logical question of the utility of their existence were not glucose phosphorylation by the glucose-6-phosphatase catalytic unit, which is sequestered within the lumen of the endoplasmic reticulum (1, 3, 4), of physiologic importance.

Studies more directly bearing on this critical metabolic question were initiated by us in 1990 wherein we combined the use of the isolated, perfused liver preparation with basic enzymology. Some of these studies were summarized in our earlier review (1). However, at that time several critical studies were unpublished, or ongoing, and were cited only as ''unpublished observations,'' by abstract reference, or as ''in press'' (see, e.g., Refs. 76 and 77 in Ref. 1). All of this work has now been completed and published as full papers (77, 86).

These findings (77, 86) indicate a directly reciprocal correlation between ureagenesis and glycogenesis from glucose in isolated livers of 48-hr fasted rats perfused with 9 or 34 m*M* glucose. The reciprocal relationship revolves about the relative availability of carbamyl-P for the urea cycle and for glucose phosphorylation (*via* carbamyl-P:glucose phosphotransferase of the glucose-6-phosphatase system) preliminary to hepatic glycogenesis. In one study (86), glutamine was shown to be more ureagenic than proline. This is because a single glutamine molecule serves as a precursor for both ammonium ion for carbamyl-P synthesis and for aspartate formation (*via* glutamate) for the urea cycle (86). A proline molecule, in contrast may provide, *via* glutamate, either an ammonium ion for carbamyl-P synthesis or an aspartate molecule for the urea cycle (85). Thus, proline is less ureagenic, and may provide for carbamyl-P synthesis for processes other than the urea cycle (e.g., for glucose phosphorylation by carbamyl-P:glucose phosphotransferase activity of the glucose-6-phosphatase system) (86).

In a second study (77), added ammonium ion, a substrate for carbamyl-P synthase I, stimulated both ureagenesis and glycogenesis in perfused livers. Norvaline, an inhibitor of ornithine transcarbamylase, the first committed step in the urea cycle, markedly reduced ureagenesis and concomitantly stimulated glycogenesis from glucose (77). Ethoxyzolamide, an inhibitor of carbonic anhydrase V, inhibited production of bicarbonate and hence biosynthesis of carbamyl-P, and inhibited both ureagenesis and glycogen-

esis. These observations, and the dependence of glycogenesis upon glucose concentration cited above, were interpreted to indicate the involvement of hepatic carbamyl-P in glucose phosphorylation preliminary to glycogenesis, as well as for urea cycle function (77, 86).

The quite surprising finding that an elevated level of glucose itself directly regulates the *in vivo* expression of the glucose-6-phosphatase catalytic unit gene independently of insulin has just been reported by the groups of Rossetti (51, 87) and Lange (19, 88). Were the sole function of glucose-6-phosphatase to increase blood glucose concentration through hydrolysis of liver glucose-6-P, elevated glucose concentration would be predicted to decrease glucose-6-phosphatase gene expression (19, 51, 87, 88). Instead, "prolonged hyperglycemia may result in overproduction of glucose *via* increased expression of this protein," as Massillon *et al.* pointed out (51).

The present authors would speculate that these new observations fit well with our proposal (1, 10, 68) that biosynthetic function of the glucose-6-phosphatase system (e.g., carbamyl-P:glucose phosphotransferase) is equally physiologically important to its more traditionally recognized glucose-6-P phosphohydrolase activity and that it may become especially metabolically important as insulin (an inducer of glucokinase gene expression) progressively decreases and blood glucose concentration (an inducer of glucose-6-phosphatase gene expression and a substrate for biosynthetic activity of the enzyme) increases. Were glucose-6-P hydrolysis the only function of glucose-6-phosphatase, the glucose-dependent induction of the glucose-6-phosphatase catalytic unit gene would be autocatalytic. An initial increase in glucose concentration would induce further glucose-6-phosphatase protein synthesis, which would hydrolyze more glucose-6-P leading to even higher levels of glucose, which would further induce formation of more glucose-6-phosphatase protein . . . To us, these considerations provide a most logical rationale for glucose phosphorylation by biosynthetic activity of the glucose-6-phosphatase system. The induction of some new glucose-6-phosphatase protein would initially lead to an elevation in blood glucose, consistent with our tuning/retuning hypothesis. However, as this level of glucose increased, so would rephosphorylation of glucose by the relatively high-K_m,glucose phosphotransferase activity of the glucose-6-phosphatase system. Thus, an elevation in blood glucose is ensured when it is most needed, but the progressive replacement of insulin-dependent glucokinase with biosynthetic activity of the glucose-6-phosphatase system would put an upper cap upon the level of glucose which may be reached. Clearly, this contrasts favorably with the alternative of unlimited blood glucose with resultant excessive loss of glucose in the urine achieved by the unconstrained, exclusively hydrolytic concept of glucose-6-phosphatase function (and is consistent with experimental observation as well).

An increase in glycogen synthase activity in isolated hepatocytes through, successively, amino acid–related hepatocyte swelling, resulting regulatory volume decrease accompanied by loss of chloride ions, consequent deinhibition of glycogen synthase phosphatase (which is inhibited by Cl^-), leading to an increase in the active form of glycogen synthase (synthase *a*), has been proposed by Meijer *et al.* (89). A parallel increase selectively in the biosynthetic function of the glucose-6-phosphatase involving these same factors recently has been reported by the present authors (90, 91). Although changes in osmolarity do not directly impact the glucose-6-phosphatase system (90, 91), Cl^- does inhibit (92). Most importantly here, the reduction in cellular Cl^- from a concentration of 30–50 m*M* to one of 10–20 m*M,* as was shown to lead to an increased glycogen synthase *a* (89), also deinhibited significantly carbamyl-P:glucose phosphotransferase, but not glucose-6-P phosphohydrolase, activity of the liver microsomal glucose-6-phosphatase system (90, 91). These parallel responses of both the biosynthetic function of the glucose-6-phosphatase system (which may initiate glycogenesis *via* glucose phosphorylation) and the glycogen synthase phosphatase/glycogen synthase system (which culminates the glycogenic process) to common stimuli through a common mechanism (Cl^- loss in response to the "regulatory volume decrease" phenomenon) lend further credibility to our hypothesis for the involvement of biosynthetic activity of the glucose-6-phosphatase system along with glucokinase in the regulated phosphorylation of glucose.

Emile van Schaftingen (93) recently employed glucosamine as an inhibitor of glucokinase within isolated hepatocytes from fed rats to study the relative contributions of glucokinase and phosphotransferase activities of glucose-6-phosphatase to glucose phosphorylation with 5 or 10 m*M* glucose present. He reported that about 12% of total glucose phosphorylation (assessed by the detritiation of [2-^{3}H]D-glucose) was resistant to glucosamine-inhibition and presumably was due to nonglucokinase enzyme activities. He attributed this activity to phosphotransferase activity of the glucose-6-phosphatase system and suggested that hexose phosphates served as phosphoryl donor(s). Nordlie, Singh, and Sukalski (94) used this same isotopic technique with hepatocytes isolated from 48-hr fasted rats (where glucokinase is lower and glucose-6-phosphatase-phosphotransferase level is higher than in fed or 24-hr fasted rats [68, 95]), and Sukalski and Nordlie (96) employed isolated, perfused livers from fed and 48-hr fasted rats, using *N*-acetylglucosamine as a glucokinase-inhibitor. They concluded the following: (i) An appreciable percentage of total hepatic glucose phosphorylation occurred by a nonglucokinase mechanism; (ii) this percentage was higher in livers from 48-hr fasted rats than from fed controls; (iii) the percentage contribution by nonglucokinase enzyme(s) was higher at 30 or 60 m*M* glucose than at 10 m*M* glucose (94, 96), and (iv) glycogenesis *via* the "indirect pathway" could not account for the glucose to glycogen flux seen in the presence of *N*-acetylglucosamine (96). In other studies with progressively fasted rats (96), it was shown that, with 48-hr

fasted rat hepatocytes incubated with 60 m*M* glucose, maximally no more than half of the glucose phosphorylation above and beyond that which could be attributed to glucokinase was due to glucose-6-P:[2-^{3}H]-glucose exchange catalyzed by glucose-6-phosphatase. This exchange reaction is irrelevant with the nonisotopic, chemical techniques we employed with isolated perfused livers (96; see also Ref. 82).

Jungermann and his group (97) have published extensively regarding the zonation of glucokinase and glucose-6-phosphatase in liver. The former is present predominantly in the perivenous region while the latter is present mainly in the periportal region (97). This places the bulk of the enzymes for glucose phosphorylation and glucose-6-P hydrolysis remote from one another, which would reduce total hepatic glucose/glucose-6-P cycling extensively and would favor net glucose utilization in the perivenous region and net glucose production in the periportal region (97). Recent work of Jonges *et al.* (98), however, indicates significant differences in K_m,glucose-6-P values as well as V_{max} for glucose-6-phosphatase (catalytic unit) within the two zones. A consequence of this is that, "if . . . glucose-6-P is . . . more or less homogeneously distributed throughout the liver lobule" (97), "metabolic flux *via* glucose-6-phosphatase would be similar in periportal and pericentral (perivenous) zones of the lobulus" (98). This new observation may have important impacts upon the tuning/retuning of blood glucose proposed here. Kinetic studies of the biosynthetic activities of the glucose-6-phosphatase system resident in the two cellular domains, although technically difficult, seem strongly indicated. The presence of this activity, along with residual glucokinase, in the perivenous region of the liver cell is consistent with its function in glucose phosphorylation.

We recognize that most of the evidence cited above, much of it our own, supportive of biosynthetic function of the glucose-6-phosphatase system in hepatic glucose phosphorylation, has come from *in vitro* studies with isolated organelles, isolated perfused livers, or isolated hepatocytes. This is the level on which we have chosen to work. Supplemental, confirmatory studies at the *in vivo* level are needed.

Recent and Some Earlier, Relevant Work with Pancreatic Islet Cell Glucose-6-Phosphatase

Pancreatic islet glucose-6-phosphatase was not considered in our earlier review (1), but is relevant here. Reports in the literature indicating the presence in pancreatic tissues of "glucose-6-phosphatase" detected by both histochemical and biochemical analyses spanning the period from 1960 to 1970 were reviewed by Nordlie (99). Generally, it could not be deduced from these studies whether the specific, "true" glucose-6-phosphatase system was involved, or if glucose-6-P hydrolysis was due to non-specific acid or alkaline phosphatases. Scott and Jones (100) reported the presence of PP_i-glucose phosphotransferase along with accompanying glucose-6-P phosphohydrolase activity in bovine pancreatic islet cells in an abstract published in 1970, thus establishing the presence there of the "true" multifunctional glucose-6-phosphatase. They suggested a role for the enzyme in glucose-sensing, based on its multifunctional nature (100). In a 1976 review, Nordlie (99) described this finding and expanded upon the potential of the multifunctional system in glucose-sensing in pancreatic islet cells. (At that time, glucokinase had not yet been identified in pancreatic islet β cells).

In 1975, Colilla, Jorgenson, and Nordlie (101) demonstrated the presence of carbamyl-P:glucose phosphotransferase activity associated with glucose-6-P hydrolysis in calf pancreatic homogenates and established that the activity was due to the glucose-6-phosphatase system. Studies by Wolf and associates (102) in 1986 demonstrated "true" glucose-6-phosphatase in isolated permeabilized rat pancreatic islet cells. In contrast with these findings, Giroix *et al.* (103) reported in 1987 that there is no specific glucose-6-phosphatase activity in pancreatic islet cells. Subsequent studies have proved the latter group wrong. In a landmark paper in 1988, Waddell and Burchell (104) prepared microsomes from isolated pancreatic islet cells from rat, mouse, rabbit, and sheep, and demonstrated the presence therein of "true" glucose-6-phosphatase by both kinetic and immunological measurements. Although glucose-6-phosphatase activity, expressed on a units/g wet tissue basis, is low in pancreas compared with liver or kidney (101), Waddell and Burchell (104) showed that in isolated pancreatic islet microsomal preparations the specific activity is high—indeed, 3.4-fold higher than that in liver microsomal preparations. They further calculated that, presuming that glucose-6-phosphatase is present exclusively in microsomes derived from pancreatic islet β cells, the specific activity would be 10-fold higher than in liver-derived microsomes. Finally, they showed that pancreatic glucose-6-phosphatase activity under optimal conditions is much higher than glucokinase, also thus determined (104). This last observation is consistent with the situation in liver (68).

Several very recent studies also confirm the presence of the glucose-6-phosphatase system, as well as glucokinase, in pancreatic islet β cells. Khan, Hong-Lie, and Landau (105) described glucose-6-phosphatase activity in permeabilized or sonicated islets from mouse pancreas as well as in microsomes therefrom, and attributed an increase in glucose/glucose-6-P cycling (106) in islets of *ob/ob* mice, and its stimulation by the glucocorticoid dexamethasone, to increased glucose-6-phosphatase activity.

The hypothesized involvement of glucokinase as the critical transducer of glucose-sensing by pancreatic islet β cells has been analyzed by Matschinsky (107, 108) in two recent papers. The role of glucose-6-phosphatase was also considered (108). Very recently, Newgard and his collaborators (109) overexpressed glucokinase and hexokinase I in isolated islet cells. They concluded that such overexpressed glucokinase must interact with other factors within the cell before becoming catalytically active (109).

Several reports have appeared recently indicating that heterozygous mutations, either naturally occurring (110–114) or induced by transgenic knock-outs (79, 115, 116) in pancreatic β cell glucokinase, are associated with a form of non–insulin-dependent diabetes mellitus termed maturity onset diabetes of the young (MODY) in human patients. For example, Froguel *et al.* (110) have shown that about 45% of individuals who have a heterozygous mutation in the pancreatic glucokinase gene exhibit overt diabetes, 22% show impaired glucose tolerance, and the remainder show a minimally elevated fasting blood glucose.

The demonstration of glucokinase along with multifunctional glucose-6-phosphatase in pancreatic islet cells, and the development of moderate to extensive hyperglycemia with genetic defects in this glucokinase, prompted us to consider the extrapolation of the fundamental features of our "tuning/retuning" concept from liver to pancreatic islet β cells, and to propose that the regulatable ratio of phosphotransferase activity of glucose-6-phosphatase/glucokinase activity, may be a critical determinant in glucose-sensing preliminary to insulin release in β cells, as it is proposed to be in hepatic glucose uptake/release. This concept is expanded upon in the last section of this review.

Recent Work with Multifunctional Glucose-6-Phosphatase of Small Intestinal Mucosa and Kidney

Glucose-6-phosphatase levels are high in small intestinal mucosa (117) and in kidney (118). However, definitive roles for activities of the enzyme in metabolism in these tissues have not yet been established. Thirty years ago, the senior author and his students (118) speculated upon roles for both the hydrolytic and biosynthetic functions of the enzyme in glucose transport *via* a "group translocation" mechanism analogous to the "PEP phosphotransferase system" of Roseman and Kundig (119). Similarities in catalytic characteristics of the glucose-6-phosphatase-phosphotransferase system and glucose transport in isolated hepatocytes (99, 120), including substrate-specificity, inhibitability, and sensitivity to phlorizin (70, 117, 118, 121), featured prominently in arguments in support of this hypothesis (121). This hypothesis is in need of reexamination in terms of modern concepts of glucose transport, glucose transporters, and glucose-6-phosphatase topology and function.

Glucose-6-phosphatase of mucosa of the small intestine remains a relatively uninvestigated entity which is deserving of more attention. No new studies surfaced in review of the literature since 1993. Of relevance in the present context: (i) The multifunctional enzyme is present in the mucosa of epithelial cells of small intestine of rat and rabbit (117); (ii) its true activity is masked by an endogenous inhibitor or inhibitors (117); and (iii) while its contribution to total glucose-6-phosphatase activity has not been quantitatively assessed, it is potentially large because of the large mass of the small intestine.

Several new reports concerning the kidney enzyme have appeared recently, in contrast with the intestinal enzyme. A very thorough study of the enzyme in developing human kidney, involving immunohistochemical techniques, has been described by Hume, Bell, Hallas, and Burchell (122). In confirmation of the work of others who used different techniques, these workers found that proximal tubules were richest in glucose-6-phosphatase; however, other tubular elements and collecting ducts were also immunopositive for glucose-6-phosphatase although at lower reactivities (122). Possible roles of glucose-6-phosphatase in individual cell types or tubular components including roles in glycogen turnover or in transcellular glucose transport as proposed by Nordlie (70) were suggested (122).

The same group in Ann Burchell's laboratory (123) used anti-$T_2\beta$ translocase antibodies and showed that, in contrast with the liver where fetal development of PP_i transport (i.e., $T_2\beta$ function) is delayed relative to the emergence of active catalytic unit (15, 123), kidney PP_i transport (via $T_2\beta$ translocase) develops concomitantly with the catalytic unit during fetal development. Voice *et al.* (124) have presented evidence that the catalytic unit and T_1, the putative glucose-6-P transport unit, may be regulated individually and differentially by, for instance, thyroxine administered to normal, hypophysectomized, and adrenalectomized rats. These observations may be related temporally to different functions of the several activities of the glucose-6-phosphatase system of the two organs during embryologic development.

A role for protein kinase C has been proposed to downregulate glucose-6-phosphatase in the kidney (125), an observation worthy of further study. Possibly related to these observations are the different temporal responses of the kidney and liver enzyme to prolonged fasting which recently has been described by Minassian and Mithieux (126). In 1965, Arion and Nordlie (127) demonstrated that hepatic glucose-6-phosphatase activity increased progressively with fasting up to a maximum at 48 hr, and then progressively declined towards the normal, fed value. Minassian and Mithieux (126) confirmed this finding, and have shown that in contrast with the liver enzyme, activity of the glucose-6-phosphatase in the kidney increases progressively during a 96-hr (i.e., 4-day) fast. These responses involve the catalytic unit, because with both tissues the noted responses were obtained with both untreated and disrupted microsomes (126, 127). Normally, liver is considered the dominant gluconeogenic tissue because of its levels of critical enzymes and its much greater mass compared with kidneys. However, Minassian and Mithieux (126) suggest "that the differential expression of glucose-6-phosphatase activity in the liver and the kidney during long-term fasting could have an important role in the shift from a principally hepatic gluconeogenesis to a hepatic and renal gluconeogenesis." In his recent review, Mithieux (8) indicates that in prolonged fasting, the kidney may account for more than 40% of total endogenous glucose production, and suggests that

the above-described observations of Minassian and Mithieux (126) may underlie this situation. Other interpretations are possible, however.

A Proposed Integrated, Coordinated Mechanism Involving Hydrolytic and Biosynthetic Activities of the Glucose-6-Phosphatase System of Liver, Pancreatic Islets, Kidney, and Small Intestinal Mucosa in Glucose Homeostasis

We first proposed in 1974 (1, 69, 70, 128) our tuning/retuning mechanism involving biosynthetic activity of the hepatic glucose-6-phosphatase system along with its hydrolytic function and hepatic glucokinase in the regulation of blood glucose concentration normally (''tuning'') and in the readjustment (''retuning'') of this parameter under pathologic or other unusual circumstances. We have since that time explained the concept rather briefly in several places (1, 10, 68). Many other factors, of course, impact upon mammalian glucose homeostasis, and it is not our intention here to imply otherwise. Our focus is upon the glucose-6-phosphatase system, however, and it is within this defined forum that we present here. The remainder of this review is an expansion of these concepts to include other, nonhepatic tissues containing the glucose-6-phosphatase system which are also intimately involved in blood glucose homeostasis.

Because a knowledge of the basics of the tuning/retuning concept as proposed for the liver is essential to the understanding of the remainder of this review, we will quote from its predecessor (1), where we very briefly summarized the main points of the hypothesis:

> The concept rests upon a controlled but adjustable balance between hepatic glucose phosphorylation and glucose-6-P hydrolysis as critical determinants of the direction and net rate of flux of glucose between the blood and the liver cell. Tradition has it that glucose phosphorylation is catalyzed by hepatic glucokinase and glucose-6-P hydrolysis by glucose-6-phosphatase. Recognizing that the former enzyme's activity is very low or absent in many species and that it is critically dependent upon insulin as inducer, we propose that biosynthetic function of glucose-6-phosphatase (Reactions 2 and 3) may act supplementary to (or in place of) glucokinase for hepatic glucose phosphorylation as circumstances may dictate (69,128). A gradual replacement of glucokinase with increasing amounts of carbamyl-P:glucose phosphotransferase activity of glucose-6-phosphatase (insulin is a repressor here [but see an earlier section of this review for a more current view]) as diabetes becomes increasingly pronounced would serve to raise progressively and continuously ambient blood glucose levels, directly proportional to the severity of the insulin insufficiency [i.e., directly proportional to the increase in blood glucose level]. This is because the K_m,glucose for phosphotransferase activity of glucose-6-phosphatase (~40 m*M*) is several-fold greater than that for glucokinase (6–12 m*M*). A progressive increase in the ratio of the high K_m enzyme/lower K_m enzyme (i.e., carbamyl-P:glucose phosphotransferase/glucokinase) as diabetes develops progressively ''retunes'' the liver to maintain increasingly higher ambient blood glucose levels (1).

Critical to the above concept and to the arguments to follow, under conditions (as with the glucose-6-phosphatase system) where K_m value >> physiologic [substrate], the activities of an enzyme with multiple substrates (e.g., the glucose-6-phosphatase system with glucose-6-P and carbamyl-P) behave kinetically independently—that is, as though reactions with the two substrates were catalyzed by two totally independent enzymes (129). The recent findings of multiple translocase T_2 forms with differing phosphate substrate specificity (15, 130), and directional specificity (25, 26) for carbamyl-P, PP_i, and P_i also are kinetically relevant.

Multifunctional glucose-6-phosphatase has earlier been identified in relatively high amounts in kidney (118) and mucosal cells of the small intestine (117) as well as in liver. More recently, the presence of the multifunctional enzyme has been demonstrated in pancreatic islet cells (101), with a specific activity in microsomes derived therefrom exceeding that in liver (104). All of these organs and tissues, in their own unique fashions, contribute to and impact upon blood glucose in health and disease. We believe that the presence in all these tissues of highly active, multifunctional glucose-6-phosphatase together with specific roles for each of these organs in overall body glucose homeostasis is more than coincidental. For that reason, we will expand our tuning/retuning hypothesis which we first proposed for liver to incorporate activities of the glucose-6-phosphatase system present also in pancreatic islets, kidney, and mucosal cells of the small intestine and to include glucokinase in our considerations of liver and pancreatic islet cells. Recent experimental observations, reviewed here, provide support for this unified concept. We will show how these concepts fit into an integrated, coordinated, regulated package contributing importantly, we believe, to the maintenance of blood glucose levels normally, and to the readjustment (''retuning'') of these levels, as the organism's needs dictate, under other than ''normal'' circumstances.

The Integrated, Coordinated Mechanistic Hypothesis. The complete glucose-6-phosphatase system is present most prominently in four tissues/organs, all of which contribute importantly to blood glucose and/or its regulation—liver, pancreatic islet β cells, kidney proximal tubule cells, and small intestinal mucosal cells. Two of these, liver and pancreatic islet β cells, contain glucokinase; and two others, kidney and small intestine, do not. Two (liver and kidney) are gluconeogenic in contrast with intes-

tinal mucosal cells and pancreatic islets. One, the pancreatic islet β cell, releases the hormone insulin in a glucose (glucose-6-P)-concentration–dependent fashion. Two of these organs, kidney and intestinal mucosa, are critical in glucose absorption/reabsorption.

We propose here that all of this may fit together as an integrated, coordinated, tightly regulated system of critical importance, along with other factors, in body glucose homeostasis (Fig. 5). Normally, the systems within the four tissues may work together, along with other enzymes and metabolic determinants, to help maintain blood glucose within rather narrow, defined limits (131). Equally importantly and equally intriguingly, this multienzyme, multiorgan system readjusts, as supranormal conditions dictate, to raise (or lower) plateau levels of blood glucose to meet the ambient needs of the organism. This may be through increased or decreased release of glucose either derived from glycogen stores (liver), through glucose release *via* gluconeogenesis (liver and kidney), through accelerated glucose uptake and storage as glycogen (liver), through glucose-concentration–dependent release of insulin (pancreatic islet β cells), or possibly through glucose absorption/reabsorption (kidney and intestinal mucosa).

Functions of Enzymes of Pancreatic Islet β Cells in the Integrated, Coordinated Scheme. The tuning/retuning hypothesis as it applies to enzymes of the liver is outlined in detail above. We will incorporate into the generalized concept activities of the glucose-6-phosphatase system together with glucokinase in pancreatic islet β cells here. Subsequently, activities of the glucose-6-phosphatase

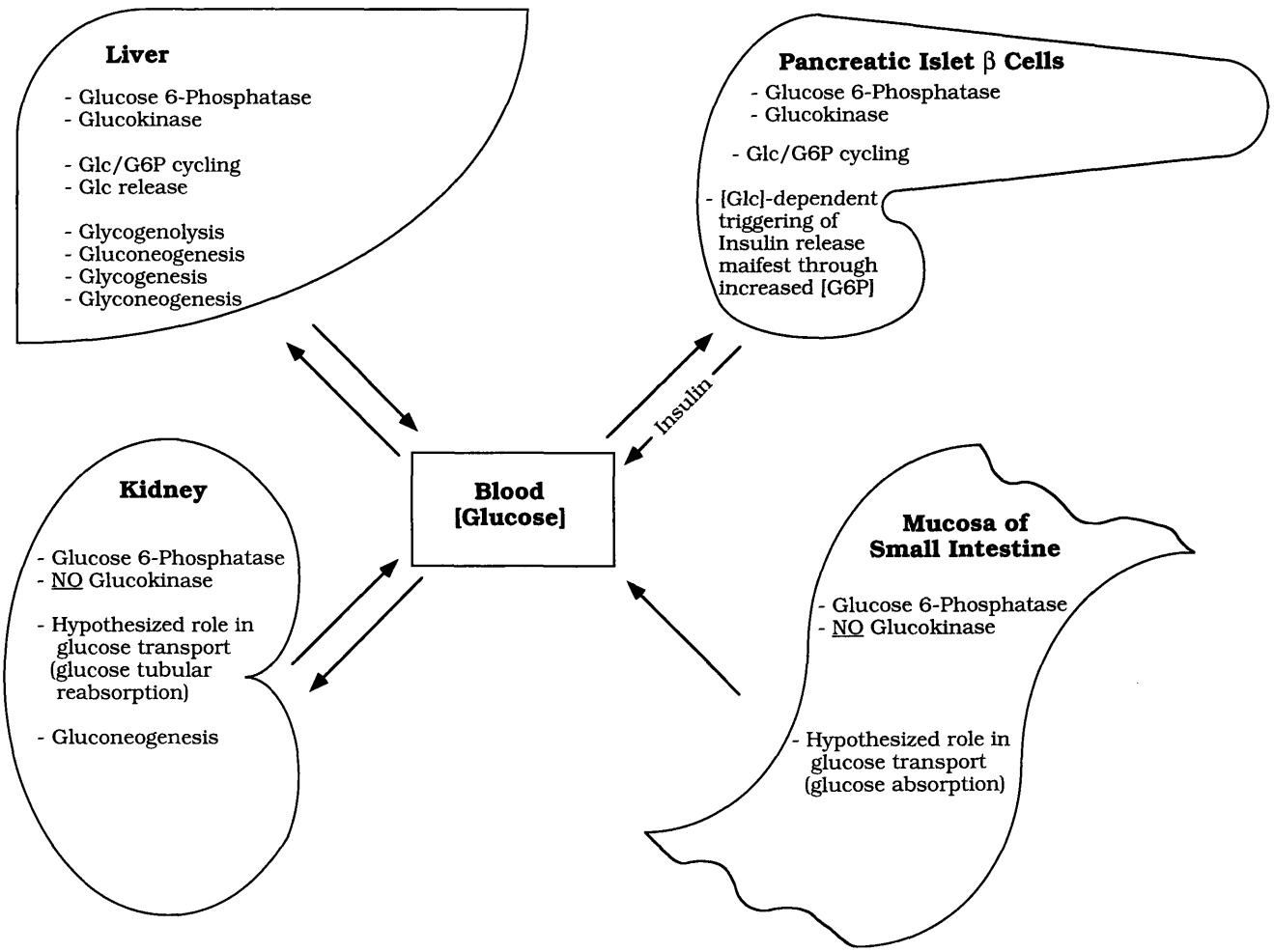

Figure 5. An integrated, coordinated mechanism involving hydrolytic and biosynthetic activities of the glucose-6-phosphatase system of liver, pancreatic islet β cells, kidney tubules, and small intestinal mucosa in body glucose homeostasis. Glucokinase, present in liver and pancreatic β cells but not in kidney or small intestine, also is incorporated. A regulatable balance between glucose-6-P hydrolysis and glucose phosphorylation *via* a combination of glucokinase and biosynthetic activity of the glucose-6-phosphatase system is proposed to "tune" the liver for net glucose production or uptake. A similar regulatable balance between glucose-6-P hydrolysis and formation *via* glucose phosphorylation is proposed to regulate the concentration of glucose-6-P in β cells and thus impact their release of insulin in response to a varied glucose load. Changes in the glucokinase/biosynthetic activity of glucose-6-phosphatase ratio constitutes a critical part of the concept related to liver and pancreas. Transport of glucose *via* a group translocase mechanism involving glucose-6-P formation and hydrolysis is proposed for kidney tubules and small intestinal mucosal cells. A role for the biosynthetic function of the glucose-6-phosphatase system in the absence of glucokinase in the kidney is also proposed. All of these factors are suggested to impact upon net blood glucose concentration under normal, basal conditions, and to modify this parameter in a continuous manner as altered conditions dictate. Additional details are given in the text.

system of kidney tubule and small intestinal mucosa will be proposed as well. With β cells of the pancreatic islets, we envision the situation to be as follows, based on recent experimental evidence summarized above:

It is recognized that the concentration of glucose-6-P in islet β cells is a critical determinant in triggering insulin release (107, 108, 132). We propose that a balance between glucose phosphorylation and glucose-6-P hydrolysis (i.e., glucose/glucose-6-P cycling [105, 106]) is critical in establishing the steady-state concentration of glucose-6-P in islet β cells, as in liver (1, 10, 68–70, 83). In that type of diabetes where β-cell glucokinase is congenitally (MODY) (110–114) or through transgenic manipulation (79, 115, 116) low or absent, we propose that biosynthetic activity of the glucose-6-phosphatase system becomes the dominant glucose-phosphorylative mechanism in β cells. Because the latter has a larger K_m,glucose than does glucokinase, the result is a shifting of the glucose-concentration–dependency curve (i.e., in a plot of glucose phosphorylation rate against glucose concentration) "to the right," (i.e. to higher concentrations of glucose) to achieve the same steady-state [glucose-6-P] which is found in the β cell under "normal" conditions.

Because β-cell glucose-6-P concentration is central in glucose-concentration–monitoring and in subsequent glucose-dependent insulin release (107, 108, 132, 133), this insulin-release in response to unit glucose-6-P is achieved at higher ambient glucose in this type of diabetes than normal (133). Thus, the tuning/retuning mechanism involving β-cell glucose-6-phosphatase-phosphotransferase (along with or without glucokinase) complements the comparable modification in liver enzyme pattern to achieve and maintain a new, higher-than-normal, steady-state blood glucose concentration in this developing diabetic state.

The major difference in the two tissues is that liver has its glucose-homeostatic function because it can take up or contribute to the blood glucose directly by glycogenesis/glycogenolysis and glycolysis/gluconeogenesis while the β cells participate through their glucose-concentration–dependent release of insulin. In developing diabetes, the liver enzymes are "retuned" to achieve a hyperglycemic steady-state blood glucose; the β-cell enzymes are likewise "retuned" to secrete insulin only in response to this newly tuned, increased blood glucose level, and hence to contribute to its continued maintenance at this elevated value.

Functions of Activities of the Glucose-6-Phosphatase System of the Kidney and Intestinal Mucosa in the Integrated, Coordinated Scheme. The intestinal mucosal cell glucose-6-phosphatase system fits into the integrated, coordinated scheme based on its potential for involvement in glucose transport in this glucose-absorptive tissue as pointed out above. The demonstrated abundance of the glucose-6-phosphatase in kidney proximal tubules where glucose reabsorption is maximal (134), also fits with the hypothesized role for activities of this enzyme in glucose transport. However, this concept is in need of further, updated experimental exploration.

The very recent observation of Minassian and Mithieux (126) that in prolonged fasting kidney glucose-6-phosphatase continues to increase linearly for at least 96 hr, while that of liver peaks at 48 hr and then declines, may be of further significance regarding activities of multifunctional glucose-6-phosphatase of kidney in blood glucose homeostasis. We believe that these differential responses of glucose-6-phosphatase in these two tissues combined with the normal presence (liver) or absence (kidney) of glucokinase in the two tissues are important in their overall roles in glucose homeostasis. Normally, glucokinase "tempers" the ultimate plateau blood glucose level to which the liver system is "tuned" because it has a smaller K_m,glucose value than does phosphotransferase activity of the glucose-6-phosphatase system (see above). Thus, when present, glucokinase makes its major contribution to glucose phosphorylation at a lower glucose than does phosphotransferase activity of glucose-6-phosphatase. In contrast with liver, the kidney system is not complicated by the glucokinase-catalyzed phosphorylation of glucose, and hence is normally "tuned" to achieve ultimately a higher plateau level of blood glucose than its liver counterpart.

With the application of these principles, the different temporal responses of the glucose-6-phosphatase system in liver and kidney (126) make metabolic sense within our overall unified, coordinated scheme. Liver, with its relatively much larger mass, normally maintains glycogen stores as a major buffering reservoir of glucose for the body. This is not so with kidney, with its comparatively much smaller mass. Thus, in a prolonged fast the kidney is designed to "go all the way" in producing glucose *via* gluconeogenesis. When the kidney glucose-6-phosphatase system remains high (glucokinase is absent), the kidney is "tuned" to achieve maximal glucose output (*via* its glucose-6-P phosphohydrolase activity along with elevated glutaminase and PEP carboxykinase activity) with minimal rephosphorylation of glucose until the concentration of blood glucose, as reflected in the kidney filtrate, is increased again to normal or above-normal levels (as in refeeding).

Regulatory Advantages of the Tuning/Retuning Mechanism Involving Glucokinase and Activities of Glucose-6-phosphatase. The tuning/retuning hypothesis, involving critically the adjustable activity ratio, biosynthetic function of glucose-6-phosphatase/glucokinase, offers the metabolic advantages of great sensitivity to even small variations in blood glucose levels over a broad range from the normal to the extensively hyperglycemic. It is not a matter of "either/or," as, for example, normoglycemia "or" pronounced untreated diabetes mellitus. Rather, the system offers a contiuum of response, adjusting to various extents of glycemia ranging from the subnormal and normal through all stages of developing diabetes mellitus to the full-blown diabetic condition. Extents of response are directly correlated with the degree of developing

insulin insufficiency or insulin insensitivity. Roles in establishing control of blood glucose in moderately elevated glycemia as seen normally in birds (69, 135) and in the aged (69) fit as well into this mechanism for control of blood glucose.

In Conclusion. Although our efforts to integrate activities of the glucose-6-phosphatase and glucokinase (where present) in the four organs/tissues most instrumental in body glucose homeostasis may have some rough edges, we believe that the many features that seem to fit provide credibility to the concept. We hope its presentation here will stimulate further research efforts in this old-fashioned but still exciting and important area of biochemistry/physiology.

Retrospective

At the end of our 1993 review (1), we suggested a number of future directions for glucose-6-phosphatase research. It is gratifying to see that in the short span of three years, studies in many of these areas are now well underway, both by old hands and researchers recently entering this challenging field.

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