Allosteric Regulation of Glycogen Synthase in Liver

A PHYSIOLOGICAL DILEMMA

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Glycogen synthase catalyzes the transfer of the glucosyl moiety from UDP-glucose to the terminal branch of the glycogen molecule and is considered to be the rate-limiting enzyme for glycogen synthesis. However, under ideal assay conditions, i.e. 37 °C with saturating concentrations of UDP-glucose and the activator, glucose-6-P, the maximal catalytic activity of glycogen synthase was only 78% of the in vivo glycogen synthetic rate. Using concentrations of UDP-glucose and glucose-6-P likely to be present in vivo, the rate was only ~30%. This prompted us to reassess a possible role of allosteric effectors on synthase activity. Glycogen synthase was assayed at 37 °C using dilute, pH 7.0, buffered extracts, initial rate conditions, and UDP-glucose and glucose-6-P concentrations, which approximate those calculated to be present in total liver cell water. Several allosteric effectors were tested. Magnesium and AMP had little effect on activity. P₃, ADP, ATP, and UTP inhibited activity. When a combination of effectors were added at concentrations approximating those present in cell water, synthase activity could account for only 2% of the glycogen synthetic rate. Thus, although allosteric effectors are likely to be playing a major role in regulating synthase activity in liver cells, to date, a metabolite that can stimulate activity and/or overcome nucleotide inhibition has yet to be identified. If such a metabolite cannot be identified, an additional or alternative pathway for glycogen synthesis must be considered.

It generally is accepted that the reaction catalyzed by glycogen synthase is rate-limiting for glycogen synthesis in all organs. This enzyme is a multiphosphorylated protein and is highly regulated through a phosphorylation-dephosphorylation mechanism. Its activity also is likely to be regulated by allosteric effectors and by the concentration of the substrate, UDP-glucose. Most, but not all, glycogen synthase is tightly bound to glycogen (1, 2). Thus, it also is possible that alterations in the binding affinity to glycogen could affect the catalytic rate.

Recently, we have become interested in the issue of in vivo synthase activity for two reasons. First of all, we have dem-onstrated that the rate of glucosyl addition to glycogen in liver from a 24-h-fasted rat given an oral glucose or fructose load (3, 4) exceeded the activity of glycogen synthase R assayed at 30 °C (or 37 °C) and at saturating concentrations of UDP-glucose and of the allosteric stimulator, glucose-6-P (5). Second, we have determined that the rate of glycogen synthesis in liver of alloxan diabetic rats given fructose orally was essentially normal, even though the activation of synthase was greatly impaired. That is, the conversion of synthase D (the most phosphorylated form) to synthase R (a less phosphorylated form) was very small (6). The impaired activation was not unexpected since we had previously reported a reduction in synthase phosphatase activity in liver from diabetic rats and a conformational change in the synthase D such that it was a poor substrate for the phosphatase (7). The lack of correlation between synthase activation, synthase R activity, and the glycogen synthetic rate was a surprise.

These data taken as a whole suggest that allosteric effectors are likely to be as important in regulating glycogen synthesis as is activation by covalent modification. In addition, it is possible that an allosteric stimulator, not yet identified, may be present and may account for the greater in vivo rate of glucosyl addition compared with the maximal rate in vitro when saturating concentrations of UDP-glucose and glucose-6-P are used. Therefore, we have begun a survey to determine potential allosteric effectors of synthase activity in vivo. Our initial results indicate a greater problem in explaining the in vivo glycogen synthetic rate than expected.

MATERIALS AND METHODS

ütC-Labeled glucose was obtained from Du Pont-New England Nuclear. UDP-14C[glucose was prepared by a method developed in our laboratory (8). Sodium secobarbital (Seconal) was obtained from Eli Lilly, Indianapolis, IN. Other high grade chemicals were obtained from Sigma. Rabbit liver glycogen was purified by passage through a mixed bed ion exchange resin (Amberlite MB-3).

Male Sprague-Dawley rats, 160–220 g, obtained from Bio-Lab, White Bear Lake, MN, were housed in a temperature (22 °C) and light (12-h cycle)-controlled animal room. Animals were fed Purina rat chow (Ralston Purina, St. Louis, MO) ad libitum until 24 h before the start of the experiment at ~6:30 a.m. The 24-h-starved animals were then gavaged with a 50% glucose solution, 4 g/kg, 20 min before they were killed. Control animals received an equivalent volume of water. Approximately 5 min after gavage, the rats were intraperitoneally anesthetized with 50 mg of Seconal/kg of body weight. At 20 min after glucose or water administration, an abdominal incision was made quickly, and the liver was immediately clamped in situ with liquid nitrogen-cooled aluminum clamps designed in our laboratory for this purpose. Only well anesthetized, noncyanotic animals were used. The liver tissue then was wrapped in aluminum foil and placed in liquid nitrogen until assayed later the same day. The rats were killed by removing the heart.

The tissue was pulverized in a liquid nitrogen-cooled mortar and
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peste. It was transferred to ice-cold Potter-Elvehjem tubes and homogenized with a motor-driven pestle in 9 volumes of 10 mM EDTA, 100 mM KF, pH 7.0 (w/w, 1:9). The tissue extract was filtered through cheesecloth and immediately assayed for synthase activity. We have shown previously that proteolysis of the synthase molecule does not occur under these circumstances. Small molecular weight species are not seen on SDS-gel electrophoresis using either P-labeled synthase or Western blotting. Addition of a mixture of proteolytic enzyme inhibitors also did not result in a change in molecular weight species obtained.

For routine assay, a filtered 1:9 tissue extract was used, and the conditions were as described previously, i.e. "total" synthase activity was assayed at 30 or 37 °C using a final concentration of 6.7 mg/ml rabbit liver glycogen, 34 mM Tris, 13 mM EDTA, 17 mM KF, 7.6 mM UDP-glucose, 7 mM glucose-6-P, pH 8.5, and a 10-min incubation. Synthase R activity was measured at pH 7.0 in the same reaction mixture except the Tris buffer was replaced with imidazole buffer, and glucose-6-P was absent. The specific activity of the UDP-glucose in the test mixture was ~60,000 cpm/mg (5). Synthase R has a broad pH optimum including a physiological pH of 7.0. Synthase D has a narrow pH optimum of ~8.5. It has little activity at pH 7.0. To assay for synthase R activity in the presence of potential allosteric effectors, an aliquot of the filtered extract was diluted further in a solution consisting of 56 mM imidazole, 0.1 mM EDTA, 1.1 mM KF, pH 7.0. Synthase R activity was assayed at pH 7.0 on the 1:100 dilution of tissue extract using a reaction mixture containing either 0.4 mM UDP-glucose and 0.2 mM glucose-6-P or 0.6 mM UDP-glucose and 0.5 mM glucose-6-P. Incubations were carried out under initial rate conditions for 2 min at 37 °C since low concentrations of UDP-glucose and glucose-6-P were used. Also, we were concerned that added metabolite concentrations could change due to metabolism. This required a very high specific activity test mixture (~9 × 10^6 cpm/μmol UDP-glucose).

The concentration of effectors added was calculated to approximate that present in the intracellular water fraction (ICF) of the liver assuming a uniform distribution. We previously determined that the ICF of the liver was 0.54 or 54% of the wet weight (9, 10). This was calculated using the following data. 1) The total liver water was 70% of the liver by weight. This did not change in fasted, fed, insulin-treated, or diabetic rats. 2) The long vascular (extracellular fraction) was determined using [35S]Na2SO4. 3) The ICF then was calculated as the total liver water minus the extracellular fraction water. That the ICF is ~54% agrees with other reports in the literature (11). When planning experiments to determine the effect of metabolites on the glycogen synthase R activity, the concentration of metabolite in liver/grown wet weight (gww) determined in our laboratory was divided by 0.54 to obtain an approximation of the concentration present intracellularly. When data were unavailable from our laboratory, literature values were used (12).

We realize that possible compartmentation of metabolites is a confounding variable. Also, the water of hydration of proteins, glycogen, and other hydrophilic macromolecular substances may limit solute diffusion in this water (13). However, we considered use of concentrations based on a uniform dilution in ICF water to be the best approximation we could use at present. Data are presented as the mean ± S.E. Statistics were determined using Student's t test for unpaired variates.

RESULTS

When assayed at 37 °C, pH 7.0, in the presence of a near-saturating concentration of UDP-glucose (7.6 mM), the synthase R activity was 0.38 ± 0.03 μmol/min/gww in the liver from 24-h-starved rats (n = 38). It was increased to 0.98 ± 0.05 μmol/min/gww in starved animals given glucose (n = 37) (2.6-fold increase). Glucose-6-P lowers the Km, for UDP-glucose. It does not affect the enzyme velocity at a saturating concentration of UDP-glucose and, thus, is not used in assaying maximal synthase R activity.

When synthase R from 24-h-starved rats was assayed at 37 °C at a physiological concentration of UDP-glucose (0.6 mM), the activity was 0.033 ± 0.010 μmol/min/gww (n = 20). Addition of a high physiological concentration of glucose-6-P (0.3 mM) increased the activity to 0.330 ± 0.057 μmol/min/gww (n = 20), a 0.2-fold increase. In 24-h-starved rats given 4 g/kg glucose orally and killed 20 min later, the activity without glucose-6-P was 0.088 ± 0.013 μmol/min (n = 36). A physiological concentration of glucose-6-P increased the activity to 0.638 ± 0.035 μmol/min/gww (n = 36). This increase was 4.2-fold. Thus, the concentration of glucose-6-P in vivo is likely to be very important in determining synthase activity. The 20-min time point was selected because we have previously shown it represented the greatest activation of synthase (3).

In rats the same dose of glucose, the glycogen synthetic rate was approximately 1.25 μmol of glycosyl units added to glycogen/min/gww (3). After fructose administration, it reached 1.50 μmol/min/gww (4). Thus, the synthase R activity when assayed at physiological concentrations of substrate and activator (glucose-6-P) and at a physiological pH could only account for about 25% of the velocity of glycosyl unit additions to glycogen in vivo. At a saturating concentration of UDP-glucose, it could account for about 65-78%.

Total (R + D) synthase activity, measured at pH 8.5, was 1.63 ± 0.06 μmol/min/gww (n = 37). This was only modestly greater than the observed rate of glycogen synthesis in vivo.

The rate of glycogen synthesis as well as the enzymic activity based on the concentration in intracellular water can be determined by dividing by 0.54. This increases both rates but does not change their relationship.

Effect of Added Metabolites—Since synthase activity in the presence of estimated intracellular concentrations of UDP-glucose and glucose-6-P could not account for the rate of glucose addition to glycogen, explanations for this were sought. The most likely was the presence of allosteric modifiers of synthase activity.

Magnesium and inorganic phosphate (P) had been reported previously (14, 15) to increase synthase activity in liver extracts, whereas ATP inhibited it (16, 17). Therefore, the effects of magnesium, P, and ATP were studied. Magnesium concentrations varying from 1.5 to 3 mM in excess of the EDTA concentration had little effect on activity in extracts from either 24-h-starved or 24-h-starved animals given glucose. This was the case whether or not (Table I) glucose-6-P was present. Inorganic phosphate (0.2–0.8 mM) inhibited activity in all of the preparations as did ATP. The latter was a very potent inhibitor even in the presence of a 1 mM excess of magnesium, i.e. 1 mM in excess of the ATP and EDTA present. An I50 of 1.0 mM was estimated. Synthase was nearly completely inhibited at a concentration of 5 mM, an approximate tissue concentration (Fig. 1). When formal kinetics were done, ATP had little effect on the Ks, for UDP-glucose but reduced the Vmax (Table II).

When 2 mM P, 1 mM ATP, and 4 mM MgCl2 were added in combination, there was little effect on the relatively low synthase R activity in the extracts containing only 0.6 mM UDP-glucose. In the presence of added 0.3 mM glucose-6-P, there was a strong inhibition, and the effects of P, and ATP were not additive (Table I).

Glucose added at concentrations within the physiological range had little effect on activity in the absence of glucose-6-P. In the presence of glucose-6-P, it inhibited activity, presumably through competition with glucose-6-P binding. Addition of ATP further inhibited activity (Table I).

Fructose-1-P has been reported to be a competitive inhibitor of phosphorylase a activity (18), and it accumulates in the hepatocyte when fructose is given orally or intravenously. Very high and unphysiological concentrations occur with enteral fructose administration (19), with fructose added to
the perfusate in perfused liver experiments (20), and are likely to be present in persons with fructose intolerance (21). In rats as well as humans, the concentrations following ingested fructose are considerably lower but are still elevated. Therefore, we tested the effect of two concentrations of fructose-1-P, 1.0 and 12 mM. These are concentrations likely to be present after oral (6) or intravenous administration of fructose (19). Synthase activity was inhibited in a concentration-dependent fashion (Table I).

We subsequently determined the effect on synthase activity of a number of potential effectors at approximate tissue concentrations. Dilute liver extracts from glucose-gavaged rats were used. UDP-glucose and glucose-6-P were present at concentrations of 0.4 and 0.2 mM, respectively. Adenosine had little effect on activity, P, again inhibited, AMP had little effect, and ADP as well as ATP inhibited activity. UTP at an estimated tissue concentration also inhibited activity. As with ATP, inhibition was noncompetitive (Table II).

When a combination of these was added together in association with a 3.4 mM excess magnesium concentration, synthase activity was reduced to a very low level (Table III). In other experiments, 1.0 and 13.0 µM fructose 2,6-bisphosphate, a regulator of flux through the glycolytic and gluconeogenic pathways, had no effect on activity (Table III). Various combinations of fructose 2,6-bisphosphate and/or fructose-1-P with other effectors also did not significantly alter the response to the effectors (data not shown).

**DISCUSSION**

In earlier studies the effects of ATP, ADP, AMP, P, and Mg on liver synthase activity have been confusing. This is because of differences in enzymic preparations used and because of different methods used to define "activated" or "inactivated" synthase (synthase a and synthase b), i.e. various states of phosphorylation of the enzyme. In addition, the conditions for assay of the synthase activity varied considerably.

ATP at various concentrations was reported to be inhibitory of the synthase activity before incubation of a liver extract but not after incubation (16, 17). Incubation was used to convert synthase to a less phosphorylated form by endogenous synthase phosphatase activity. Later, others reported inhibition of activity of all forms of the enzyme (22, 24). However, addition of inorganic phosphate was reported to relieve ATP inhibition (22).

P, has been reported to stimulate synthase activity and to replace glucose-6-P as an allosteric stimulator (14). Later, De

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**TABLE I**

| Activity (glucose-gavaged rats) | % |
|--------------------------------|---|
| 0.6 mM UDP-glucose (28)*       | 100 ± 13 |
| +1.5 mM MgCl₂ (12)             | 90 ± 11  |
| +3.0 mM MgCl₂ (12)             | 108 ± 26 |
| +1 mM ATP, 2 mM MgCl₂ (4)      | 65 ± 19  |
| +5 mM ATP, 6 mM MgCl₂ (4)      | 18 ± 3*  |
| +2 mM P, 3 mM MgCl₂ (4)        | 39 ± 11* |
| +2 mM P, 1 mM ATP, 4 mM MgCl₂ (4) | 79 ± 10 |
| +0.2 mM Pi (4)                 | 67 ± 8   |
| +4.0 mM Pi (4)                 | 70 ± 6   |
| +8.0 mM Pi (4)                 | 58 ± 14  |
| +10 mM glucose (4)             | 95 ± 17  |
| +20 mM glucose (4)             | 106 ± 12 |
| +10 mM glucose, 5 mM ATP, 6 mM MgCl₂ (4) | 32 ± 2* |
| 0.6 mM UDP-glucose + 0.3 mM glucose-6-P (30)* | 100 ± 6 |
| +1.5 mM MgCl₂ (8)              | 75 ± 17  |
| +3.0 mM MgCl₂ (8)              | 117 ± 16 |
| +1 mM ATP, 2 mM MgCl₂ (6)      | 64 ± 15  |
| +5 mM ATP, 6 mM MgCl₂ (4)      | 8 ± 1*   |
| +2 mM P, 3 mM MgCl₂ (6)        | 52 ± 21  |
| +2 mM P, 1 mM ATP, 4 mM MgCl₂ (5) | 42 ± 9* |
| +0.2 mM Pi (4)                 | 82 ± 14  |
| +4.0 mM Pi (4)                 | 61 ± 7*  |
| +8.0 mM Pi (4)                 | 63 ± 11* |
| +10 mM glucose (4)             | 60 ± 4*  |
| +20 mM glucose (4)             | 59 ± 6*  |
| +10 mM glucose, 5 mM ATP, 6 mM MgCl₂ (4) | 5 ± 1*  |
| +1 mM F1P (4)                  | 53 ± 3*  |
| +12 mM F1P (4)                 | 30 ± 2*  |

* Mean activity, 0.057 µmol/min/g, wet weight.

**FIG. 1. Effect of ATP concentration on synthase R activity.** Rats were gavaged with glucose, 4 g/kg, 20 min before they were killed. Liver synthase activity was determined in the presence of 0.6 mM UDP-glucose (UDPG), 0.3 mM glucose-6-P (G6P), and varying concentrations of ATP. Incubation was carried out for 2 min at 37 °C. The I₅₀ for ATP was approximately 0.8 mM. n = 4 for each data point.
Rats used is shown in parentheses. The muscle enzyme in contrast to a rather potent inhibition competitive in type. Of all the metabolites tested, only glucose-6-P was stimulatory. Glucose itself was modestly inhibitory in the presence of glucose-6-P. It did not reverse the inhibition by ATP or the inhibition by ATP in glucose-6-P. It did not reverse the inhibition by ATP or the inhibition by ATP in glucose-6-P. The inhibition by ATP in glucose-6-P could not fully explain the rate of glycogen synthesis observed, just as in the present study the synthase activity was only about 62% of the glycogen synthetic rate. When UDP-glucose, glucose-6-P, ATP, ADP, AMP, and Pi were added, their effects were not additive. Clearly, their effects were not additive. Magnesium chloride did not stimulate activity either in the presence or absence of glucose-6-P. 

0.4 mM UDP-glucose and glucose-6-P concentrations, which approximated those calculated to be present in total liver cell water. Under these conditions, Pi as well as ATP were found to be inhibitory, and their effects were not additive. Clearly, Pi could not substitute for glucose-6-P as an allosteric stimulator as reported previously (14). Magnesium chloride did not stimulate activity either in the presence or absence of glucose-6-P. It did not reverse the inhibition by ATP. ATP in the presence of an excess of magnesium was primarily non-competitive in type. Of all the metabolites tested, only glucose-6-P was stimulatory. Glucose itself was modestly inhibitory in the presence of glucose-6-P.

All of the nucleotides tested also were potent inhibitors at a concentration approximating that in vivo except for AMP. Piras et al. (25) also reported a lack of inhibition by AMP of the muscle enzyme, while a rather potent inhibition reported by Roach and Larner (26). Also, Piras et al. (25) observed strong inhibition of both the least phosphorylated and most phosphorylated forms of skeletal muscle synthase by ATP, ADP, UDP, and P, whereas Roach and Larner (26) reported strong inhibition of only the most phosphorylated forms by ATP; ADP, and P.

In our studies, when P and the nucleotides were added as a group and at estimated intracellular concentrations, synthase activity became essentially unmeasurable (Table III). To date we have been unable to identify a metabolite that reverses inhibition by the nucleotides or inorganic phosphate. Several years ago, Piras and Staneloni (27) reported that tetanic stimulation of skeletal muscle in vivo resulted in activation of phosphorylase and glycogenolysis. During the recovery phase after electrical stimulation, glycogen was rapidly resynthesized. Measurement of synthase activity in muscle extracts at a saturating concentration of both UDP-glucose and glucose-6-P could not fully explain the rate of glycogen synthesis observed, just as in the present study the synthase activity was only about 62% of the glycogen synthetic rate. When UDP-glucose, glucose-6-P, ATP, ADP, AMP, and Pi were added at physiological concentrations, the synthase rate was only 1.2% of that obtained at saturating concentrations of UDP-glucose and glucose-6-P. This also is similar to the present results in which liver enzyme activity was evaluated. In both studies, glycogen always was present in excess in the assay mixtures.

Piras and Staneloni (27) suggested compartmentalization of effectors in order to explain the poor correlation of synthase activity with in vivo glycogen synthetic rate. However, this is an unlikely explanation since skeletal muscle synthase activity in the presence of saturating concentrations of UDP-glucose and glucose-6-P was less than the glycogen synthetic rate, just as in the liver. The rate was considerably less when both the substrate and positive effector were present at physiological concentrations. Addition of the negative effectors reduced the activity to a barely detectable level.

In livers from alloxan diabetic rats, synthase R activity measured using saturating concentrations of UDP-glucose and glucose-6-P is even less than that in normal animals, and yet the glycogen synthetic rate is similar to that in the normal animals when both are given fructose (6). Thus, both in liver and in skeletal muscle, we are left with a dilemma. That is, how to explain the observed rate of glucosyl unit addition to glycogen when glycogen synthesis is very active.

It is possible that the enzyme in extracts has undergone a conformational change rendering it catalytically less efficient than in vivo and/or it has been desensitized to positive effectors. This could be due to the processing of the tissue, etc., or could be the result of the loss of a labile activator, etc. A stable in vivo activator is not likely to be playing a role, since we have not found greater synthase activity using concentrated (1:3) extracts. Nevertheless, an in vivo allosteric stimulator remains an attractive possibility.

At pH 7.0, a physiological pH for the liver, synthase D, the most phosphorylated form of synthase, has little activity even in the presence of saturating concentrations of UDP-glucose substrate and the modifier glucose-6-P. It has a rather narrow pH optimum with maximal activity at pH 8.5 (5). Thus, this form of the enzyme presumably has little catalytic activity in vivo. Identification of another potent effector that stimulates synthase D activity at pH 7.0 or below potentially could help explain the glycogen synthetic rate in vivo, but then the regulation of synthase activity through a phosphorylation-

**TABLE II**

| Effect of inhibitors on estimates $K_{m}$ and $V_{max}$ |
|-----------------------------------------------|
| $0.3$ mM glucose-6-P and MgCl$_2$ at a concentration equal to the ATP and UTP present in all assay mixtures was used. Number of rats used is shown in parentheses. |
| $K_{m}$ UDP-glucose | $V_{max}$ |
|-----------------------------------------------|
| Base line (9) | $0.09 \pm 0.01$ | $1.10 \pm 0.13$ |
| 0.5 mM ATP (3) | $0.09 \pm 0.01$ | $0.69 \pm 0.12$ |
| 0.4 mM UTP (2) | $0.10 \pm 0.01$ | $0.58 \pm 0.01$ |

**TABLE III**

| Synthase activity (glucose-giaved rats) |
|---------------------------------------|
| Number of rats used is shown in parentheses; EDTA concentration, 1.0 mM. |
| Activity | $\mu$mol/min/gew |
|---------------------------------------|
| 0.4 mM UDP-glucose + 0.2 mM glucose-6-P (14) | $0.415 \pm 0.052$ |
| +0.11 mM adenosine (8) | $0.424 \pm 0.035$ |
| +8 mM Pi (6) | $0.289 \pm 0.044$ |
| +0.4 mM AMP (6) | $0.594 \pm 0.044$ |
| +3 mM ADP (6) | $0.073 \pm 0.011^a$ |
| +6 mM ATP (8) | $0.011 \pm 0.002^a$ |
| +0.5 mM UTP (6) | $0.285 \pm 0.040$ |
| +1 mM F$_2$, 6-bis-P (6) | $0.349 \pm 0.050$ |
| +13 mM F$_2$, 6-bis-P (6) | $0.344 \pm 0.055$ |
| 0.6 mM UDP-glucose + 0.3 mM glucose-6-P (4) | $0.490 \pm 0.083$ |
| +10 mM glucose, 3 mM ATP, 1 mM ADP, 0.3 mM AMP, 0.2 mM UTP, 3.0 mM Pi, 4.0 mM MgCl$_2$ (4) | $0.027 \pm 0.006^a$ |

* Statistically different from control.

Wulf et al. (22) reported stimulation by Pi, only of the a form of the enzyme. The latter was defined as enzymic activity present after pretreatment of animals with glucose. Subsequently, others (23) found Pi inhibition of synthase a activity, not stimulation. Hizukuri and Larner (16) reported inhibition of synthase activity by high concentrations of ADP and AMP as well as by ATP before incubation of an extract but not afterward. Magnesium has been reported to stimulate synthase activity in general (15), but others found only stimulation of that form of the enzyme present before incubation of a liver extract (22). Although allosteric modification of synthase activity has been demonstrated previously, it is difficult to compare data presented here with data reported previously. Different enzyme preparations, substrate concentrations, and pH values were used in the assays. Also, the conditions used previously often make it difficult to determine which forms of the enzyme were being assayed.

In the present experiments, synthase was assayed at 37 °C using dilute, pH 7.0, buffered extracts, initial rate conditions, UDP-glucose and glucose-6-P concentrations, which approximate those calculated to be present in total liver cell water. Under these conditions, Pi, as well as ATP were found to be inhibitory, and their effects were not additive. Clearly, Pi could not substitute for glucose-6-P as an allosteric stimulator as reported previously (14). Magnesium chloride did not stimulate activity either in the presence or absence of glucose-6-P. It did not reverse the inhibition by ATP or the inhibition by ATP added with Pi. The inhibition by ATP in the presence of an excess of magnesium was primarily non-competitive in type. Of all the metabolites tested, only glucose-6-P was stimulatory. Glucose itself was modestly inhibitory in the presence of glucose-6-P.

All of the nucleotides tested also were potent inhibitors at a concentration approximating that in vivo except for AMP. Piras et al. (25) also reported a lack of inhibition by AMP of the muscle enzyme in contrast to a rather potent inhibition.
dephosphorylation mechanism would lose significance (26). The latter is unlikely.

It also is possible that an alternate pathway for glycogen synthesis is operative in vivo. Phosphorylase activity exceeds synthase activity by 10-fold in liver (28). The phosphorylase-mediated reaction is readily reversible. Thus, the possibility that it may function synthetically under some in vivo conditions should at least be considered (29). The substrate concentrations and pK values should favor glycogenolysis. However, if even a small amount of activity in the synthetic direction is present, it would help explain the discrepancy in rates.

Recently, NMR studies have suggested significant liver glycogen turnover in vivo following glucose (or fructose) administration to rats. That is, there is a degree of futile cycling when glycogen is accumulating (30). If this is the case, then the difference between the catalytic activity of glycogen synthase and the actual rather than the net glycogen synthesis rate observed becomes even more difficult to explain. It is clear that there is much to be learned regarding the regulation of glycogen synthase activity and its relationship to the increase in in vivo glycogen mass following the ingestion of carbohydrates.

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