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# Cellular Concentrations of Enzymes and Their Substrates

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The activity of crude and pure enzyme preparations as well as the molecular weight of these enzymes were obtained from the literature for several organisms. From these data enzyme concentrations were calculated and compared to the concentration(s) of their substrates in the same organism. The data are expressed as molar ratios of metabolite concentration to enzyme site concentration. Of the 140 ratios calculated, 88% were one or greater, indicating that in general substrates exceed their cognate enzyme concentrations. Of the 17 cases where enzyme exceeds metabolite concentration, 16 were in glycolysis. The data in general justify the use of enzyme kinetic mechanisms determined *in vitro* in the construction of dynamic models which simulate *in vivo* metabolism.

## Introduction

The actual structure of the cell's internal environment has been a topic of debate for many years and new ideas about this organization have appeared frequently in the recent scientific literature. Rather than enzymes and metabolites mixing randomly in a dilute aqueous environment, a more structured organization clearly exists. There is evidence which suggests that interactions between the cytosolic enzymes of a metabolic pathway may lead to the direct channeling of metabolites between these enzymes (Davis, 1967; Koch-Schmidt *et al.*, 1977; Leu & Kaplan, 1970; Nover *et al.*, 1980; Srivastava & Bernhard, 1986*a*,*b*). Other soluble enzymes have been found bound to cellular substructures, for example, the association of glycolytic enzymes with the contractile apparatus (Dustin, 1984) or the association of tricarboxylic acid cycle enzymes in mitochondria (Robinson & Srere, 1985). Thus cellular compartmentation exists at the level of organelles or macrocompartments as seen in mitochondria and lysosomes, and possibly at the level of proteins or microcompartments where localized areas of high protein concentrations form an interacting system.

One of the consequences of high protein concentration, in addition to the formation of microcompartments, is that the concentration of an enzyme may be higher than the concentration of its substrate(s) (Ottaway & Mowbray, 1977; Sols & Marco, 1970; Srere, 1967, 1968; Srivastava & Bernhard, 1986*a*). Srivastava & Bernhard (1986*b*) compared the concentrations of some of the glycolytic enzyme sites of mammalian muscle tissue with the concentration of the related intermediary metab-

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olites and found several enzymes whose active site concentration exceeded the substrate concentrations. This observation led them to conclude that metabolites are probably passed directly from one enzyme site to another without dissociating to a free-state. Therefore, these cytosolic enzymes possibly form metastable complexes *in vivo.*

This new "structural-functional level of organization" (Friedrich, 1984) is of great relevance to the construction and analysis of dynamic models purporting to simulate metabolism *in vivo* (El-Rafai & Bergman, 1976; Heinrich *et al.*, 1977; Kohn *et al.*, 1977; London, 1966; McMinn & Ottaway, 1976; Wright & Kelly, 1981). These models incorporate enzyme mechanisms and kinetic constants determined under the usual in vitro conditions using the classical Michaelis-Menten assumptions. One of these assumptions is that the free substrate concentration is equal to the total substrate concentration; that is, the concentration of enzyme-substrate complex is much smaller than substrate concentration. However, when substrate is of comparable concentration to enzyme, in a perfectly mixed system, a high percentage of metabolite would be bound to enzyme and free metabolite would be lower than total concentration. It has been suggested that the excess of high affinity enzyme binding sites compared to substrate concentration usually occurs in other metabolic pathways as well as in the muscle glycolytic system (Srivastava & Bernhard, 1986*b*).

## Literature Survey

In this paper, the general conclusion that enzyme site concentration exceeds metabolite concentration was examined. The literature for six organisms was surveyed for: (a) enzyme activity in crude fractions, (b) enzyme activity of purified fractions, (c) enzyme molecular weight, and (d) the concentration of pertinent metabolites from the same sources. The enzyme active-site and substrate concentrations were calculated and the data expressed as a molar concentration ratio of substrate : enzyme active site. When the literature reported a number of values, for example, for enzyme activity in crude extracts, we biased our selection to favor the conclusions of Srivastava & Bernhard (1986*b*).

The enzymes and metabolites used in this survey were from glycolysis and related carbohydrate metabolism, the pentose-phosphate pathway, amino acid metabolism, the glyoxylate, urea, and Calvin cycles. The six organisms employ different metabolic strategies and needless to say, the available literature in part determined the choices of enzymes and organisms. *Escherichia coli* was chosen as a model for prokaryotes. It has a great variety of cytosolic proteins and metabolities, lacks extensive macrocompartmentation, and thrives in an aqueous medium from which it acquires all its nutrients. In yeast cells, the cytosolic protein fraction consists largely of glycolytic enzymes and macrocompartmentation is present. Mammalian muscle cells also contain large quantities of glycolytic enzymes in the cytosol, and in addition many proteins are associated with the contractile apparatus. Liver cells function in many areas of metabolism and probably contain the greatest variety of proteins. Red blood cells exist in a relatively aqueous environment, have little intracellular macrocompartmentation and contain a high concentration of a specialized protein,

hemoglobin. *Dictyostelium discoideum*, a cellular slime mold, is unusual in that it uses protein as its major energy source throughout differentiation. These systems, covering three of the Kingdoms of the biological world, provide a fairly wide spectrum of the metabolic variation found in today's living organisms.

### Calculation of Enzyme and Metabolite Concentrations

Enzyme concentrations were calculated as follows: (a) turnover number was calculated from the specific activity (μmol substrate converted $min^{-1}$ mg $protein^{-1}$ of the most purified enzyme fraction available using the molecular weight of the holoenzyme and assuming that all protein represented active holoenzyme; the turnover number was expressed as μmol substrate converted $min^{-1}$ μmol $enzyme^{-1}$); (b) a $V_{max}$ value, assumed to represent *in vivo* activity, was calculated from the specific activity of a crude enzyme fraction using the conversion factors listed in Table 1; $V_{max}$ values were expressed as μmol substrate converted $min^{-1}$ liter cell

TABLE 1

*Protein concentration and water content of different cell types*

| Source | Water content (g 100 $g^{-1}$ moist tissue) | Protein (mg $ml^{-1}$ cell volume)‡ | Reference |
|---|---|---|---|
| *E. coli* | 70 | 235 | Ingraham *et al.* (1983) |
| Yeast | 65 | 280 | Altman & Dittmer (1964) |
| *D. discoideum* | 70 | 121 | Walsh & Wright (1978) |
| Rat liver | 69 | 313 | Long (1961) |
| Rat muscle | 77 | 260 | Long (1961) |
| Human RBC† | 65 | 158 | Long (1961) |
| Pig heart | 77 | 260 | Long (1961) |

† Red blood cells.
‡ Calculated based on mg protein-$mg^{-1}$ wet weight.

$volume^{-1}$; (c) enzyme concentration was determined by dividing the $V_{max}$ value from (b) by the turnover number from (a). The total enzyme site concentration was finally determined by multiplying enzyme concentration by the number of subunits per holoenzyme, assuming one active site per subunit. If a specific polymer size were required for catalysis then the enzyme concentration was modified by the appropriate factor. The final calculated value is referred to as the enzyme site concentration, expressed as μM and compared to μM substrate concentration(s). The calculated ratios are given in Table 2.

In an ideal analysis all metabolite and enzyme data would come not only from the same laboratory, but also from the same tissue and from the same extract. Unfortunately, these circumstances are rarely reported in the literature. However, it was possible to obtain the data for most of our calculations from the same organism or tissue; exceptions are noted. In some cases, if not available from the organism in question, the enzyme molecular weight and/or number of subunits per holoenzyme

TABLE 2

*Substrate concentration ($\mu M$), enzyme site concentration ($\mu M$)*

| Carbohydrate metabolism | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Rat | Rabbit[j] | Rat | Human |
| Enzyme | Substrate | *E. coli* | Yeast | D.d.[c] | Liver | Muscle | Muscle | RBC |
| $(Glc)_n$ Syn | $(Glc)_n$[a] | | | 1036[dc] | 22 900 | 123 | | |
| | UDPG | | 461 | 100[dc] | 206 | 8 | | |
| $(Glc)_n$ | $(Glc)_n$ | | | 670 | | | 15[k] | |
| Phosphorylase | Pi | | 8800<br>1279[h] | 588-5882 | | | 54 | |
| Glc kinase | Glc | | | 10[d] | 13 500 | | | |
| | ATP | 3 | | 13[d] | 4 840 | | | |
| HK | Glc | | | | | | 5057 | |
| | ATP | | 91-137 | | | | 6540 | |
| PGM | Glc1P | | 529 | | | 84[l] | | |
| PGI | F6P | | 8 | 1[dc] | | 11[l] | | 140 |
| PFK | F6P | | 48 | 30[d] | | 8[l] | | 333 |
| | ATP | 278<br>236[h] | 82-142 | 30[d] | | 72 | | 34 000 |
| Aldolase | FDP | | 8-21 | | 2 | 0·9[l] | 0·1 | 10 |
| TPI | DHAP | | 14 | | | 0·4[l] | | 9 |
| GA3PD | GA3P | | 7-21 | | | 0·02[l] | | |
| | NAD | 19-32[e] | 18 | | | 0·3 | | |
| | Pi | | 393 | | | 5 | | |
| GPGA kinase | 3PGA | | 5 | | | 0·4[l] | 0·7 | 48 |
| | ATP | 70 | 9 | | | 27 | 48 | 1 130 |
| PGA mutase | 3PGA | | 3 | | | 0·2[l] | | |
| Enolase | 2PGA | | | | 2 | 0·02[l] | 0·04[l] | |
| PK | PEP | 5-18<br>4-13[h] | 0·2 | | 8 | 0·1[l] | 0·1 | 17 |
| | ADP | 50<br>35[h] | 5 | | 101 | 14 | 19 | 183 |
| F1,6 bis-P'tase | FDP | | 708-1875 | 0·08[d] | 0·8 | 2[l] | | 0·9 |
| G6PD | Glc6P | | 657 | | 53-87 | | | 771 |
| | NADP | | 6-43 | | 22 | | | 628 |
| 6PGD | 6PG | | 6-19 | | | | | 19 |
| | NADP | | 5 | | | | | 55 |
| Gal 1P UT | Gal 1P | | | | | | | |
| | UDPG | 54 | 316 | | | | | |
| Gal kinase | Gal | | | | | | | |
| | ATP | 21-36 | | | | | | 807 |

| Protein metabolism | | | | |
|---|---|---|---|---|
| Enzyme | Substrate | *E. coli* | Yeast | Rat liver |
| Asp TA | Asp | 20-64 | 600-2600 | 30-48 |
| | $\alpha$KG | 22 | 40-1000 | 6 |
| Glu D | Glu | 15 780 | 7890-18 420[d] | |
| | NADP | | 10-79[e] | |
| Gln Syn | Glu | 665 | | 144-164 |
| | $NH_3$ | | | 28-32 |
| | ATP | 101 | | 147-167 |

TABLE 2—continued

| Protein metabolism | | | | |
|---|---|---|---|---|
| Enzyme | Substrate | *E. coli* | Yeast | Rat liver |
| Glu decarboxylase | Glu | 112 | | |
| Ser dehydrase | Ser | | | 367-417 |
| GSSG reductase | GSSG | | | |
| | NADPH | | 10-32[e] | 270 |
| Orn decarboxylase | Orn | | 256 | |
| Carbamoyl P Syn | Gln | | | 147-208 |
| | ATP | 550 | | 101 |
| Aspartase | Asp | 5-18 | | |

| Miscellaneous | | | | |
|---|---|---|---|---|
| | Metabolic | | | |
| Enzyme | System | Pathway | Substrate | Ratio |
| UDPG pyrophosphosphorylase | D.d. | CHO[g] | UDPG | 69[c] |
| | | | PPi | |
| Transaldolase | Yeast | CHO | F6P | 0·8 |
| | | | E4P | 10[b] |
| Malic enzyme | Rat muscle | TCA[h] | Malate | 107 |
| | | | NAD(P) | |
| Tyr TA | Rat liver | Prot[i] | Tyr | |
| | | | $\alpha$KG | 9 |
| Gln Syn | Rat muscle | Prot | Glu | 382-430[m] |
| | | | $NH_3$ | 65-73 |
| | | | ATP | 569-640 |
| GSSG reductase | Human RBC | Prot | GSSG | 96 |
| | | | NADPH | |
| Asp transcarbamoylase | *E. coli* | Prot | Asp | 5-16 |
| | | | Carbamoyl P | |
| Glycerol kinase | *E. coli* | | Glycerol | |
| | | | ATP | 27 |
| Uridine phosphorylase | D.d. | CHO | Uridine | |
| | | | Pi | 384-3850[d,e] |
| | Rat liver | CHO | Uridine | |
| | | | Pi | 480-560 |
| PEP carboxylase | *E. coli* | CHO | PEP | 12-40 |
| Pyr carboxylase | Yeast | CHO | Pyr | 320[d] |
| | | | ATP | 220-320[d] |
| | Rat liver | CHO | PYR | 25 |
| | | | ATP | 478 |

[a] Assumed 1 site per 12 glucose units.
[b] Second enzyme form.
[c] *Dictyostelium discoideum.*
[d] Assumed molecular weight.
[e] Assumed number of subunits.
[f] Metabolite concentrations were from rat muscle.
[g] CHO: carbohydrate.
[h] TCA: tricarboxylic acid cycle.
[i] Prot: protein.
[j] rabbit hind-limb and and back muscles.
[k] whole rat muscle.
[l] unspecified skeletal muscle.
[m] rat hind-limb muscles.

*Abbreviations*: *Substrates*: $(Glc)_n$: glycogen; UDPG: uridine diphosphate glucose; Pi: inorganic phosphate; Glc: glucose; ATP: adenosine triphosphate; Glc1P: glucose-1-phosphate; F6P: fructose-6-phosphate; FDP: Fructose, 1,6-bisphosphate; DHAP: dihdroxyacetone-phosphate; GA3P: Glyceraldehyde-3-phosphate; NAD: nicotinamide dinucleotide; 3PGA: 3-phosphoglycerate; 2PGA: 2-phosphy-

*continued overleaf*

glycerate; PEP: phosphoenolpyruvate; ADP: adenosine diphosphate; Glc6P: glucose-6-phosphate; NADP: nicotinamide dinucleotide phosphate; 6PG: 6-phosphogluconate; Gal 1P: galactose-1-phosphate; Gal: galactose; Asp: aspartate; Glu: glutamate; Ser: serine; GSSG: oxidized glutathione; NADPH: reduced nicotinamide dinucleotide phosphate; orn: ornithine; Pyr: pyruvate; GIP: guanosine triphosphate; PPi: pyrophosphate; R5P: ribose-5-phosphate; E4P: erythrose-4-phosphate; Tyr: tyrosine; carbamoyl P: carbamoyl phosphate.

*Enzymes*: $(Glc)_n$ Syn: glycogen synthase; HK: hexokinase; PGM: phosphoglucomutase, PGI: phosphoglucoisomerase; PFK: phosphofructokinase; TPI: triose phosphate isomerase; GA3PD: glyceraldehyde-3-phosphate dehydrogenase; PGA Mutase: phosphosphoglycerate mutase; PK: pyruvate kinase; Fl, 6 bisP'tase: fructose 1, 6-bisphosphatase; G6PD: glucose-6-phosphate dehydrogenase; 6PGD: 6-phosphogluconate dehydrogenase; Gal 1P UT: galactose-1-phosphate uridyl-transferase; Asp TA: aspartate transaminase; Glu D: glutamate dehydrogenase; Gln Syn: glutamine synthase; Carbamoyl P Syn: carbamoyl phosphate synthase; PDC: pyruvate dehydrogenase complex; Tyr TA: tyrosine transaminase.

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from a different but closely related organism were used in the calculation of turnover number ([d] and [e] in Table 2). This appeared to be justified since the molecular weights are rather consistent among closely related organisms. Enzyme activities from crude extracts or purified enzyme (used in calculating turnover number) were always from the organism stated.

The metabolite concentrations were usually obtained from the literature in units of $\mu mol\ g^{-1}$ of dry or fresh weight and converted to $\mu M$ using the data given in Table 1. As mentioned above, metabolite and enzyme data were also obtained from the same organism unless otherwise noted. The major exception is for the muscle metabolites. Muscle metabolite concentrations were from rat muscle whereas most of the enzyme data were obtained from rabbit. Where enzyme data for rats was available, the ratios were similar to those reported for the rabbit data, implying that enzyme activities from these tissue types were similar. Moreover, it was interesting to note that metabolite concentrations were more consistent than the enzyme concentrations between the various organisms. This may, in part, reflect the fact that substrates are relatively stable compared to enzymes, and can be recovered in comparable amounts even though different methodologies are used. The concentrations of substrates and enzyme active sites are expressed in terms of cell volume (i.e. water content). We chose what we judged as the best values for total water content of the various systems to determine both the metabolite and enzyme site concentrations. However, as both concentrations were determined using the same water content, the ratios are not dependent upon the accuracy of these values.

There are of course, many sources of error which may affect these ratios. In general, cellular integrity, organelles and metabolic compartments must be destroyed in order to measure either enzyme activity or metabolite concentrations. The presence of extracellular metabolites may be a problem for some concentrations reported, and result in higher concentrations than were actually present and measured intracellularly. This could be an important consideration for both muscle and liver tissues, which are difficult to wash free of extracellular metabolites. However, it would be a minor consideration for the micro-organisms, as these are easily washed free of contaminating extracellular metabolites. An additional problem is differential distri-

bution of metabolite and enzyme. In muscle tissue Hintz *et al.* (1984) found heterogeneity in both enzymes and metabolites within individual muscle fibers. They suggest larger volume extracts as the best methodology for correlating components in muscle tisue. Metabolite may exist in several intracellular compartments, for instance, cytosol and mitochondria, whereas enzyme may only exist in a single compartment. Thus concentration based on the entire cell volume may not reflect intracompartment concentrations of either metabolities or enzymes. Unfortunately, there is no general method of predicting whether the concentration of a compartmented metabolite or enzyme will be higher or lower than that based on the total cell volume. For instance, if mitochondrial volume is estimated to be one tenth of total cell volume, then 90% of a metabolite could exist extramitochondrially and intramitochondrial concentration would be the same as that calculated based on total cell volume. If only 5% were intramitochondrial, the average concentration based on cell volume would be twice that of mitochondrial concentration. However, if 15% were intramitochondrial, then concentration within the mitochondrion would be greater than that calculated based on total cell volume. If enzyme were entirely found in a smaller compartment, then its concentration would in reality be higher than that predicted based on total cell volume. Thus, if the same volume assumptions were made, and enzyme were found exclusively in the mitochondrion, then site concentration could be ten times higher than that calculated based on total cell volume.

There is also a problem of free vs. bound water. Enzymes and other proteins are generally associated with a fairly stable layer of water. This bound water does not behave as bulk water and may additionally decrease the actual volume in which metabolites are dissolved (Srere, 1985). Another source of error is an over-estimation of substrate concentration if a fraction is enzyme-bound, for example, where several competing enzymes are involved. With respect to multiple use of ATP in carbohydrate metabolism (Table 2), the individual ratios are so high that they would not be significantly affected. With respect to ADP, using the free ADP concentration, as suggested by Seraydarian *et al.* (1962), the lowest ratio we found would change from 14 to 5·6.

Errors are also involved in the measurement of enzymatic activity. Of necessity, enzymes must be diluted to abnormally low protein concentrations and frequently optimal, rather than physiological, conditions of pH and temperature are employed in their assay. Available enzyme sites may be over-estimated, as a significant fraction of the enzyme sites assayed in dilute solution may be inactive or unavailable to substrate *in vivo* due to enzyme-bound product or inhibitor or compartmentation of enzyme from substrate. For example, in muscle, a substantial part of glyceraldehyde-3-P dehydrogenase exists bound to 3-phosphoglycerate (Block *et al.*, 1971). For 23 enzymes of carbohydrate metabolism and the citric acid cycle in *Dictyostelium*, it has been possible to make a meaningful comparison between calculated *in vivo* enzyme activity and enzyme activity measured *in vitro* ($V_{max}$) (Wright & Albe, 1989). Excluding three extreme values (two of which were enzyme complexes), $V_{max}$ values were on average 30-fold higher than calculated *in vivo* enzyme activities. Thus, we would predict that available enzyme site concentration based on $V_{max}$ values in

crude extracts is in general overestimated. Enzymes have also been found to serve structural roles (Wistow *et al.*, 1987). Available enzyme sites *in vivo* may also be underestimated when measuring enzyme activity *in vitro*, due to the disruption of enzyme complexes, proteolytic inactivation, the dilution (loss) of unknown activators, and so on. A single subunit may not have an active site, be active only in a particular polymeric array, or in rare cases have multiple active sites.

Enzyme recovery from extracts may not be complete. This is of particular concern in the muscle, as many enzymes can bind to the contractile apparatus and thus be removed from the crude extract by centrifugation commonly employed to rid the extract of cellular debris (Clarke & Masters, 1975; Clarke & Morton, 1976).

## Results of Survey

The ratios in Table 2 are expressed as μM substrate/μM enzyme active site. For the total of 140 ratios calculated, 123 were one or greater, and 105 were greater than or equal to ten. Sixty-eight of the enzymes examined had calculated ratios for all substrates and 52 of these had all ratios greater than or equal to one. Therefore, it is reasonable to assume that in general the choice of metabolites did not inherently favor those which are either easily isolated or of extremely high concentration within the cell.

Glycogen concentration is usually expressed in glucose equivalents. However, this tends to overestimate the sites available to glycogen-processing enzymes. Therefore, we estimated there was one site available for every 12 glucose equivalents based on the average chain length of glycogen of 12 glucose units. Brammer *et al.* (1972) found that all but 25% of glycogen could be degraded by β-amylase. Thus, our figure may be relatively conservative in the estimation of total available sites, as we assume only 1/12 of the glycogen is available for degradation. Also, the ratios of glycogen concentration to glycogen processing enzymes are so large that even another ten-fold decrease in glycogen site availability would still result in high ratio values. A ten-fold decrease in glycogen concentration would mean that less than 1% of the glucose would be available to processing enzymes.

The majority of the cases in which enzyme site exceeds substrate concentration is in glycolysis. Of the 17 ratios less than one, 16 were in the glycolytic pathway, and of these 12 were in rabbit or rat muscle. Thus, we substantiate Svrivastava and Bernhard's observations, but find them to be almost unique to glycolysis in muscle. In general, substrate exceeds enzyme site concentration *in vivo*. Glycolytic enzymes from other sources do not show this pattern as strikingly as muscle tissue, which is highly specialized and unique in its need for a very rapid mobilization of available energy sources. The association or microcompartmentation of enzymes and substrates may have evolved in this system to insure that the majority of the substrates present were bound, making them immediately available for metabolic processing. Moreover, protein is used as an energy source, especially under nutritional stress. Due to the general vulnerability of most proteins to proteolytic attack, excessive enzyme protein concentration may be essential in order to insure adequate catalytic activity in times of stress.

## Discussion

Perhaps these compiled data will stimulate thought among those studying the in-vivo organization and metabolism of cells. New information on the organization of cytosolic proteins within cells focuses attention on a problem biochemists have struggled with since the beginning of this discipline: the extent to which in-vitro data are relevant to in-vivo metabolism. With respect to a basic assumption underlying Michaelis–Menten kinetics, the data summarized by the ratios of substrate : enzyme concentration in Table 2 would seem, in general, to justify the use of enzyme kinetic mechanisms and constants determined *in vitro* in dynamic metabolic models. For the reactions modelled in the *Dictyostelium* system, most of these ratios are greater than one. Kinetic models incorporating enzyme mechanisms represent an analytical tool with which the relevance of specific enzyme mechanisms to metabolism *in vivo* may be examined (Kelleher *et al.*, 1978; Kelly *et al.*, 1979; Wright & Kelly, 1981).

The ratios of muscle metabolites to enzyme site concentrations were unique in this analysis. It is therefore critical to examine the assumptions underlying the generated ratios to see if some bias was developed in the analysis. In general, enzyme site concentration from muscle tissues would tend to be underestimated—mainly due to incomplete extraction and inactivation of an enzyme during its isolation from crude extracts. On the other hand metabolite concentrations were probably overestimated, when based on total cellular volume. This is because contaminating extracellular metabolites were more likely to have been included in the total concentration. Other factors, such as inhomogeneous distribution of metabolites and enzymes, might also influence in-vivo metabolite to enzyme site concentration ratios. Most of these factors would tend to increase the calculated ratios, that is, the actual in-vivo ratio would be smaller. This is additional evidence that there are probably some physiologically significant differences between muscle tissue and the other cell types examined.

In Michaelis–Menten analyses, the total substrate concentration is assumed to equal the free substrate concentration. This assumption holds well for in-vitro kinetic analyses since substrate concentrations are generally in large excess compared to enzyme sites; this assumption also holds for most of the cases presented in Table 2, as the majority of the ratios are greater than ten. However, in in-vivo situations where substrate concentration may be comparable to enzyme site concentration, or where several enzymes are competing for the same substrates (Sols & Marco, 1970; Srere, 1985), a considerable portion of the substrate may be bound to the enzyme(s). Bound substrate concentrations can be calculated from a dissociation constant (Segel, 1975) or from an equilibrium constant (Sols & Marco, 1970). Free substrate concentration (total minus bound substrate) can be employed in a general Michaelis–Menten analysis to predict the actual velocity of the reaction. However, Srivastava and Bernhard have demonstrated, *in vitro*, that pairs of complementary dehydrogenases can directly transfer NAD from one to the other (Srivastava & Bernhard, 1986*a*). In this analysis the predicted rate of the reaction, based on dissociated substrate concentration, was much lower than the measured rate. Therefore, they conclude that more than the dissociated substrate was available to the enzyme. This

leads to an effectively higher intracellular or intracompartmented substrate concentration than would be predicted based on dissociation constants. Recent reanalysis of these data suggests that the original interpretation was wrong. The observed rates could instead be explained using more classical approaches and without invoking a direct transfer mechanism (Chock & Gutfreund, 1989). Coprecipitation of isolated enzymes has also been used as evidence for enzyme:enzyme interactions. These coprecipitations are specific and have been performed for a number of enzymes involved in the TCA cycle (Halper & Srere, 1977; Sumegi *et al.*, 1980; Beeckmans & Kanarek, 1981; Fahien & Kmioteck, 1983; Porpaczy *et al.*, 1983; Sumei *et al.*, 1985).

Another physical consideration which may increase the concentration of substrates is that protein may occupy a significant portion of the volume within the cell or macrocompartment. Thus, Srere (1985) has proposed that opposition of complementary enzyme sites and trapping of metabolites within a protein matrix may lead to higher concentrations than calculated based on total cell or macrocompartment volume. Kinetic models may be useful in predicting whether an enzyme participates in a direct transfer mechanism, where more than dissociated substrate concentration should be considered, or whether free substrate concentrations should be calculated by the use of dissociation constants and used in the enzyme kinetic expression to more accurately simulate conditions *in vivo.*

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## APPENDIX

### Legends for Tables AI–AIV

The values given for yeast were obtained mostly from the genus *Saccharomyces* with the exception of a few which were taken from *Candida.*

The values given for muscle were obtained mostly from rabbit muscle. The exceptions are from rat muscle and are indicated as such by a footnote.

The values given for plants were obtained from spinach leaves, unless otherwise noted.

If two values are listed for one enzyme, they refer to different enzyme forms.

*Note*: A reference for each value is given in parentheses. The references are listed at the end of the Appendix.

### TABLE AI

*The activity from a crude extract and a highly purified preparation of each enzyme is given. Activity is expressed as* $\mu mol\ min^{-1}\ mg\ protein^{-1}$

| Enzyme | *E. coli* | | Yeast | | *D. discoideum* | |
|---|---|---|---|---|---|---|
| | Crude | Pure | Crude | Pure | Crude | Pure |
| Glycogen synthetase | 0·21 (1) | 380 (1)<br>505 (1) | 0·016 (2) | 91 (2) | 0·00045 (214) | 0·22 (214) |
| Glycogen phosphorylase | — | — | 0·15 (6) | 135 (6)<br>25 (6) | 0·032 (7) | 7·14 (7) |
| UDPG pyrophosphorylase | 0·16 (10)‡ | 103 (10)‡ | — | — | 0·4 (11) | 200 (11) |
| Glucokinase | — | — | 7 (15) | 79 (15) | 0·011 (16) | 0·51 (16) |
| Hexokinase | — | — | 1·8 (18) | 800 (18) | — | — |
| Phosphoglucomutase | 0·365 (20) | 19 (20) | 0·081 (21) | 205 (21) | 0·27 (22) | — |
| Phosphoglucoisomerase | — | — | 5·7 (25) | 675 (25) | 0·2 (26) | 14·2 (26) |
| Phosphofructokinase | 0·27 (29)<br>0·36 (29) | 190 (29)<br>205 (29) | 0·3 (30) | 60 (30) | 0·085 (31) | 4·4 (31) |
| Aldolase | — | — | 4·7 (33) | 108 (33) | 0·02 (22) | — |
| Triose P isomerase | — | — | 41 (38) | 10 000 (38) | — | — |
| GA3P dehydrogenase | 0·4 (41) | 40 (41) | 3 (42) | 155 (42) | 0·46 (22) | — |
| 3PGA kinase | 0·7 (44) | 98 (44) | 26 (45) | 945 (45) | 0·42 (22) | — |
| PGA mutase | 1·7 (44) | 124 (44) | 28 (49) | 1077 (49) | 0·14 (22) | — |
| Enolase | 4·1 (50) | 147 (50) | — | 200 (51) | 0·14 (22) | — |
| Pyruvate kinase | 0·52 (53)<br>0·52 (53) | 124 (53)<br>110 (53) | 10 (54) | 340 (54) | 0·12 (22) | — |
| Lactate dehydrogenase | 0·03 (57) | 78 (57) | — | — | 0·03 (22) | — |
| Fl,6-bisphosphatase | — | — | 0·02 (58) | 73 (58) | 0·0036 (59) | 0·0202 (59) |
| G6P dehydrogenase | — | — | 0·27 (63) | 678 (63) | 0·15 (64) | — |
| 6PG dehydrogenase | 0·034 (68) | 32 (68) | 0·12 (69) | 42 (69) | 0·034 (64) | — |
| R5P isomerase | — | — | 0·39 (71) | 24 (71) | 0·26 (64) | — |
| Ru5P 3-epimerase | — | — | 0·39 (73) | 262 (73) | 0·024 (64) | — |
| Transketolase | — | — | 0·3 (75) | 43 (75) | 0·024 (64) | — |
| Transaldolase | — | — | 6 (78)<br>0·83 (78) | 61 (78)<br>44 (78) | 0·77 (64) | — |

| Rat liver | | Rabbit muscle | | Human RBC | | Spinach leaf | |
|---|---|---|---|---|---|---|---|
| Crude | Pure | Crude | Pure | Crude | Pure | Crude | Pure |
| 0·016 (3) | 35 (3) | 0·020 (4) | 12 (4) | — | — | 0·015 (5) | 3 (5) |
| — | — | 3·1† (8) | 85† (8) | — | — | 0·014 (9) | 44 (9) |
| — | — | 0·06 (12) | 82·5 (12) | 0·0078 (13) | 127 (13) | 0·096 (14)‡ | 94 (14)‡ |
| 0·01 (17) | 80 (17) | — | — | — | — | — | — |
| — | — | 0·022† (19) | 120† (19) | — | — | — | — |
| — | — | 8 (23) | 1100 (24) | — | — | — | — |
| — | — | 8 (27) | 950 (27) | 0·027 (28) | 830 (28) | — | — |
| — | — | 2·5 (23) | 180 (23) | 0·03 (32) | 136 (32) | — | — |
| 0·03 (34) | 1·51 (34) | 0·99† (34) | 13·5† (34) | 0·002 (36) | 16 (36) | 0·13 (37) | 12 (37) |
| | | 0·13 (35) | 16 (35) | | | | |
| — | — | 130 (39) | 7000 (39) | 2·4 (40) | 10 236 (40) | — | — |
| — | — | 20 (23) | 120 (23) | — | — | 0·96 (43) | 80 (43) |
| — | — | 7·2† (46) | 635† (46) | 0·2 (46) | 680 (46) | 6·6 (48) | 702 (48) |
| | | 20 (47) | 975 (47) | | | | |
| — | — | 19 (23) | 1000 (23) | — | — | — | — |
| 0·32 (52) | 87 (52) | 5·8† (52) | 120† (52) | — | — | — | — |
| | | 4·5 (23) | 80 (23) | | | | |
| 1·5 (55) | 520 (55) | 5† (55) | 380† (55) | 0·009 (56) | 330 (56) | — | — |
| | | 11 (23) | 340 (23) | | | | |
| — | — | 20 (23) | 600 (23) | — | — | — | — |
| 0·14 (60) | 34 (60) | 0·06 (61) | 22 (61) | — | — | 0·04 (62) | 62 (62) |
| | | | | | | 0·05 (62) | 109 (62) |
| 0·19 (65) | 210 (66) | — | — | 0·0025 (67) | 220 (67) | — | — |
| — | — | — | — | 0·002 (70) | 15 (70) | — | — |
| — | — | — | — | — | — | 0·78 (72) | 2171 (72) |
| — | — | 0·2 (74) | 20 (74) | — | — | — | — |
| 0·008 (76) | 1·5 (76) | — | — | 0·0008 (77) | 8·1 (77) | — | — |
| — | — | — | — | — | — | — | — |

TABLE AI—continued

| Enzyme | *E. coli* Crude | *E. coli* Pure | Yeast Crude | Yeast Pure | *D. discoideum* Crude | *D. discoideum* Pure |
|---|---|---|---|---|---|---|
| Ru,5BisP Carboxylase | N.A. | | N.A. | | N.A. | |
| GalIP uridyltransferase | 0·86 (81) | 209 (81) | 0·1 (82) | 688 (82) | — | — |
| Galactokinase | — | — | 0·75 (83) | 55·8 (83) | — | — |
| Ala transaminase | — | — | — | — | — | — |
| Asp transaminase | 1·2 (86) | 307 (86) | 0·4 (86) | 502 (86) | — | — |
| Tyr transaminase | — | — | — | — | — | — |
| Glu dehydrogenase (NAD) | — | — | 0·09 (90) | 23 (90) | — | — |
| (NADP) | 0·29 (89) | 250 (89) | 0·15 (90) | 89 (90) | — | — |
| Gln synthetase | 0·5 (91) | 90 (91) | — | — | — | — |
| Arginase | — | — | — | — | — | — |
| Glu decarboxylase | 2·3 (95) | 68 (95) | — | — | — | — |
| Ser dehydrase | 0·36 (96) | 280 (96) | — | — | — | — |
| GSSG reductase | 0·09 (98) | 320 (98) | 0·15 (99) | 153 (99) | — | — |
| Ornithine decarboxylase | 0·02 (102) | 99 (102) | 0·00033 (103) | 0·7 (103) | — | — |
| Arg decarboxylase | 0·01 (105) | 16·4 (105) | — | — | — | — |
| DAP decarboxylase | 0·035 (106) | 7·5 (106) | N.A. | | N.A. | |
| DHPA reductase | 0·16 (107) | 300 (107) | N.A. | | N.A. | |
| Asp transcarbamoylase | 8 (108) | 117 (108) | — | — | — | — |
| CarbamoylP synthetase | 0·01 (109) | 6 (109) | — | — | — | — |
| Aspartase | 1·1 (111) | 68 (111) | — | — | — | — |
| Lys decarboxylase | 23 (112) | 1000 (112) | — | — | — | — |
| 3PGA dehydrogenase | 0·018 (113) | 6·7 (113) | — | — | — | — |
| UDPG 4-epimerase | — | — | 0·2 (82) | 21 (82) | — | — |
| Glycerol kinase | 1·2 (114) | 41 (114) | — | — | — | — |
| Uridine phosphorylase | — | — | — | — | 0·013 (115) | 7·8 (115) |
| PEP carboxylase | 0·28 (117) | 88·1 (117) | N.A. | | N.A. | |
| Pyruvate carboxylase | — | — | 0·07 (119) | 30 (119) | — | — |

| Enzyme | *E. coli* Crude | *E. coli* Pure | Yeast Crude | Yeast Pure | *D. discoideum* Crude | *D. discoideum* Pure |
|---|---|---|---|---|---|---|
| PDC | 0·51 (121) | 23 (121) | 0·16 (122) | 29 (122) | 0·004 (123) | 0·93 (123) |
| Citrate synthase | 0·4 (124) | 150 (124) | 0·37 (125) | 160 (125) | 2·0 (126) | 111 (126) |
| Isocitrate dehydrogenase | 1·2 (129) | 125 (129) | 0·036 (130) | 35·6 (130) | 0·017 (131) | 2·76 (131) |
| SuccinylCoA synthetase | 0·54 (133) | 29·4 (133) | — | — | — | — |
| alpha-KGDC | 0·030 (135) | 1·5 (135) | — | — | 0·029 (136) | 3·29 (136) |
| Succinate dehydrogenase | 0·23 (212) | 1·21 (212) | — | — | 0·35 (138) | 14 (138) |
| Malate dehydrogenase | 6·4 (208) | 542 (140) | — | — | 2 (141) | 550 (141) |
| Malic enzyme | 0·1 (143) | 177 (143) | — | — | 0·016 (144) | 0·64 (144) |

† Rat muscle.
‡ ADPG pyrophosphorylase.
‖ Porcine liver.
¶ Bovine heart.
N.A. = Not applicable.

| Rat liver | | Rabbit muscle | | Human RBC | | Spinach leaf | |
|---|---|---|---|---|---|---|---|
| Crude | Pure | Crude | Pure | Crude | Pure | Crude | Pure |
| N.A. | | N.A. | | N.A. | | 0·35 (79) | 2·3 (80) |
| — | — | — | — | — | — | — | — |
| — | — | — | — | 0·00002 (84) | 0·081 (84) | — | — |
| 0·92 (85) | 501 (85) | — | — | — | — | — | — |
| 0·82 (87) | 156 (87) | — | — | — | — | — | — |
| 0·53 (88) | 267 (88) | — | — | — | — | — | — |
| — | — | — | — | — | — | — | — |
| 0·03 (92) | 8·9 (92) | 0·001† (93) | 1·09† (93) | — | — | — | — |
| 6 (94) | 5310 (94) | — | — | — | — | — | — |
| — | — | — | — | — | — | — | — |
| 0·54 (97) | 278 (97) | — | — | — | — | — | — |
| 0·069 (100) | 269 (100) | — | — | 0·0035 (101) | 165 (101) | — | — |
| 0·00005 (104) | 20 (104) | — | — | — | — | — | — |
| — | — | — | — | — | — | — | — |
| N.A. | | N.A. | | N.A. | | N.A. | |
| N.A. | | N.A. | | N.A. | | N.A. | |
| — | — | — | — | — | — | — | — |
| 0·45 (110) | 25·6 (110) | — | — | — | — | — | — |
| — | — | — | — | — | — | — | — |
| — | — | — | — | — | — | — | — |
| — | — | — | — | — | — | — | — |
| — | — | — | — | — | — | — | — |
| — | — | — | — | — | — | — | — |
| 0·002 (116) | 2·4 (116) | — | — | — | — | — | — |
| N.A. | | N.A. | | N.A. | | 0·03 (118) | 19·9 (118) |
| 0·09 (120) | 25 (120) | — | — | — | — | — | — |

| Rat liver | | Muscle | | Pig heart | | Plant | |
|---|---|---|---|---|---|---|---|
| Crude | Pure | Crude | Pure | Crude | Pure | Crude | Pure |
| — | — | — | — | 0·0007 (215) | 0·10 (215) | — | — |
| 0·09 (127) | 124 (127) | — | — | 0·35 (128) | 33 (128) | — | — |
| 0·08 (206)‖ | 45·6 (206)‖ | — | — | 0·4 (132) | 31·3 (132) | — | — |
| — | — | — | — | 0·14 (134) | 120 (134) | — | — |
| — | — | — | — | 0·003 (137) | 0·42 (137) | — | — |
| — | — | — | — | 0·6 (139) | 4·2–5·1 (139) | — | — |
| 0·08 (142) | 41 (142) | — | — | — | — | — | — |
| 0·16 (145) | 30 (145) | 0·006† (146) | 20† (146) | 0·026 (209)¶ | 10·3 (209)¶ | — | — |

## TABLE AII

*The molecular weight of the enzyme, the number of subunits, and the molecular weight of each subunit is given units of molecular weight (MW) are in* $K^{Da}$

| Enzyme | *E. coli* | | Yeast | | *D. discoideum* | |
|---|---|---|---|---|---|---|
| | MW | SU-MW | MW | SU-MW | MW | SU-MW |
| Glycogen synthetase | 93 (1)<br>200 (1) | 2-50 (1)<br>4-50 (1) | 300 (2) | 4-71 (2) | — | — |
| Glycogen phosphorylase | — | — | 250 (6)<br>390 (6) | 2-103 (6)<br>4-103 (6) | 210 (7) | 2-95 (7) |
| UDPG pyrophosphorylase | 210 (10)‡ | 4-50 (10)‡ | — | — | 200 (148) | — |
| Glucokinase | — | — | 96 (15) | 2-50 (15) | — | — |
| Hexokinase | — | — | 104 (149) | 2-52 (149) | — | — |
| Phosphoglucomutase | 62-65 (20) | — | 65 (21) | 2-32 (151) | — | — |
| Phosphoglucoisomerase | — | — | 119 (152) | 4-30 (152) | — | — |
| Phosphofructokinase | 140 (29)<br>148 (29) | 4-35 (29)<br>4-37 (29) | 835 (30) | 4-118 (30)<br>4-112 (30) | — | — |
| Aldolase | — | — | 80 (157) | 2-40 (157) | — | — |
| Triose P isomerase | — | — | 53-56 (38) | 2-26 (38) | — | — |
| GA3P dehydrogenase | 144 (41) | — | 142 (42) | 4-35 (42) | — | — |
| 3PGA kinase | 44 (44) | 1-44 (44) | 46 (48) | 1-46 (48) | — | — |
| PGA mutase | 56 (44) | — | 112 (49) | 4-27 (49) | — | — |
| Enolase | 90 (50) | 2-45 (50) | 88 (160) | 2-44 (160) | — | — |
| Pyruvate kinase | 240 (53)<br>190 (54) | 4-60 (53)<br>4-51 (54) | 210 (54) | 4-50 (54) | — | — |
| Lactate dehydrogenase | 74 (57) | 1-74 (57) | — | — | — | — |
| Fl,6-bisphosphatase | — | — | 130 (58) | 4-35 (58) | — | — |
| G6P dehydrogenase | — | — | 128 (163) | 4-N.G. (164) | — | — |
| 6PG dehydrogenase | 100 (68) | 2-50 (58) | 100 (69) | 2-50 (69) | — | — |
| R5P isomerase | — | — | 105 (165) | 4-N.G. (165) | — | — |
| Ru5P 3-Epimerase | — | — | 46 (73) | — | — | — |
| Transketolase | — | — | 159 (76) | 2-79 (76) | — | — |
| Transaldolase | — | — | 68 (78)<br>65 (78) | 2-34 (78)<br>2-32 (78) | — | — |
| Rul,5BisP carboxylase | N.A. | | N.A. | | N.A. | |
| GallP uridyltransferase | 80 (81) | 2-41 (81) | 86 (82) | 2-38 (82) | — | — |
| Galactokinase | — | — | 58 (83) | 1-58 (83) | — | — |
| Ala transaminase | — | — | — | — | — | — |
| Asp transaminase | 84 (166) | 2-43 (167) | 90 (168) | 2-45 (167) | — | — |
| Tyr transaminase | — | — | — | — | — | — |
| Glu dehydrogenase (NAD)<br>(NADP) | —<br>250 (89) | —<br>NG-40§ (89) | 350 (170) | — | — | — |
| Gln synthetase | 600 (91) | 12-50 (91) | — | — | — | — |
| Arginase | — | — | — | — | — | — |
| Glu decarboxylase | 310 (95) | 6-50 (95) | — | — | — | — |
| Ser dehydrase | 37 (96) | — | — | — | — | — |
| GSSG reductase | 105 (98) | 2-50 (98) | 118 (99) | — | — | — |
| Ornithine decarboxylase | 160 (102) | 2-81 (102) | 86 (103) | 2-NG (103) | — | — |
| Arg decarboxylase | 300 (105) | 4-74 (105) | — | — | — | — |
| DAP decarboxylase | 200 (106) | — | — | — | — | — |
| DHPA reductase | 110 (107) | — | — | — | — | — |

| Rat liver | | Rabbit muscle | | Human RBC | | Spinach leaf | |
|---|---|---|---|---|---|---|---|
| MW | SU-MW | MW | SU-MW | MW | SU-MW | MW | SU-MW |
| 260 (3) | 3–85 (3) | 250 (147) | 3–90 (149) | — | — | 69 (5) | — |
| — | — | 185† (8) | 2–N.G. (8) | — | — | 194 (9) | 2–92 (9) |
| — | — | — | — | 440 (13) | — | 210 (14) | — |
| 53 (71) | 1–53 (17) | — | — | — | — | — | — |
| — | — | 100† (19) | 1–100† (150) | — | — | — | — |
| — | — | 67 (24) | 1–67 (24) | — | — | — | — |
| — | — | 132 (27) | 2–66 (153) | 132 (154) | 2–63 (154) | — | — |
| — | — | 340 (155) | 4–83 (155) | 420 (156) | 4–104 (156) | — | — |
| 158 (34) | 4–40 (34) | 158† (34)<br>160 (158) | 4–40† (34)<br>4–40 (158) | 158 (36) | 4–40 (36) | 120 (37) | 4–30 (37) |
| — | — | 50 (39) | 2–25§ (39) | 56 (40) | 2–28 (40) | — | — |
| — | — | 145 (23) | 4–36 (159) | — | — | 150 (43) | 4–37 (43) |
| — | — | 46† (46)<br>46 (46) | 1–46† (46)<br>1–46 (46) | 45 (46) | 1–45 (46) | 46 (48) | 1–46 (48) |
| — | — | 57–64 (49) | 2–27 (49) | — | — | — | — |
| 91 (52) | 2–53 (52) | 93† (52)<br>100 (161) | 2–49 (52)<br>2–46 (161) | — | — | — | — |
| 208–220 (55) | 4–55 (55) | 250† (55)<br>240 (162) | 4–57† (55)<br>2–115 (162) | 250 (56) | 4–63 (56) | — | — |
| — | — | — | — | — | — | — | — |
| 140–176 (60) | 4–40 (60) | 144 (61) | 4–36 (61) | — | — | 130 (62)<br>160 62) | 4–33 (62)<br>4–40 (62) |
| 130 (66) | 2–65 (66) | — | — | 210 (67) | 4–53 (67) | — | — |
| — | — | — | — | 104 (70) | 2–52 (70) | — | — |
| — | — | — | — | — | — | 53 (72) | 1–53 (72) |
| — | — | — | — | — | — | — | — |
| 130 (76) | 2–65 (76) | — | — | — | N.G.–70 (77) | — | — |
| — | — | — | — | — | — | — | — |
| N.A. | | N.A. | | N.A. | | 557 (79) | 8–N.G. (79) |
| — | — | — | — | — | — | — | — |
| — | — | — | — | 55 (84) | 2–25 (84) | — | — |
| 114 (85) | — | — | — | — | — | — | — |
| 93 (87) | 2–46 (87) | — | — | — | — | — | — |
| 115 (169) | 4–32 (169) | — | — | — | — | — | — |
| — | — | — | — | — | — | — | — |
| 350–400 (92) | 8–45 (92) | 350–400§ (92) | 8–44§ (92) | — | — | — | — |
| 118 (171) | 4–30 (171) | — | — | — | — | — | — |
| — | — | — | — | — | — | — | — |
| 60–68 (97) | 2–35† (97) | — | — | — | — | — | — |
| 100 (100) | 2–50 (100) | — | — | 115 (101) | 2–56 (101) | — | — |
| 105 (104) | 2–50 (104) | — | — | — | — | — | — |
| — | — | — | — | — | — | — | — |
| — | — | — | — | — | — | — | — |

TABLE AII—continued

| Enzyme | *E. coli* MW | *E. coli* SU-MW | Yeast MW | Yeast SU-MW | *D. discoideum* MW | *D. discoideum* SU-MW |
|---|---|---|---|---|---|---|
| Asp transcarbamoylase | 220 (108) | 6 (172) | — | — | — | — |
| CarbamoylP synthetase | 163 (109) | 1-130<br>1-42 (173) | — | — | — | — |
| Aspartase | 193 (111) | 4-50 (111) | — | — | — | — |
| Lys decarboxylase | 800 (112) | 10-80 (112) | — | — | — | — |
| 3PGA dehydrogenase | 163 (113) | 4-40 (113) | — | — | — | — |
| UDPG 4-epimerase | — | — | 183 (82) | 2-78 (82) | — | — |
| Glycerol kinase | 220 (114) | 4-55 (114) | — | — | — | — |
| Uridine phosphorylase | — | — | — | — | — | — |
| PEP carboxylase | 402 (117) | 4-100 (117) | N.A. | | N.A. | |
| Pyruvate carboxylase | — | — | — | — | — | — |

| Enzyme | *E. coli* MW | *E. coli* SU-MW | Yeast MW | Yeast SU-MW | *D. discoideum* MW | *D. discoideum* SU-MW |
|---|---|---|---|---|---|---|
| PDC | 4600 (121) | 24-96 (121) | 8000 (176) | 40-45 (176)<br>40-35 | — | — |
| Citrate synthase | 280 (124) | — | — | — | 110 (126) | — |
| Isocitrate dehydrogenase | 95 (178) | 2-53 (178) | 300 (179) | 8-40 (179) | — | — |
| SuccinylCoA synthetase | 160 (181) | 2-39 (181)<br>2-30 (181) | — | — | — | — |
| alpha-KGDC | 2500 (182) | 12-95 (182) | — | — | — | — |
| Succinate dehydrogenase | 100 (212) | 1-65 (213)<br>1-25 (213) | — | — | — | — |
| Malate dehydrogenase | 61 (140) | 2-30 (140) | — | — | 70 (141) | — |
| Malic enzyme | 200 (143) | 4-54 (143) | — | — | — | — |

† Rat muscle.
‡ ADPG pyrophosphorylase.
§ Subunits are inactive.
‖ Porcine liver.
¶ Bovine heart.
†† dehydrogenase subunit only.
N.A. = Not applicable.
N.G. = Not given.

| Rat liver | | Rabbit muscle | | Human RBC | | Spinach leaf | |
|---|---|---|---|---|---|---|---|
| MW | SU-MW | MW | SU-MW | MW | SU-MW | MW | SU-MW |
| — | — | — | — | — | — | — | — |
| 316 (174) | 2–160 (174) | — | — | — | — | — | — |
| — | — | — | — | — | — | — | — |
| — | — | — | — | — | — | — | — |
| — | — | — | — | — | — | — | — |
| — | — | — | — | — | — | — | — |
| — | — | — | — | — | — | — | — |
| 103 (175) | 4–26 (175) | — | — | — | — | — | — |
| N.A. | | N.A. | | N.A. | | 560 (118) | 4–130 (118) |
| — | N.G.-130 (120) | — | — | — | — | — | — |

| Rat liver | | Muscle | | Pig heart | | Plant | |
|---|---|---|---|---|---|---|---|
| MW | SU-MW | MW | SU-MW | MW | SU-MW | MW | SU-MW |
| — | — | — | — | 3080 (215)†† | 40–41 (215)¶<br>40–36 (215) | — | — |
| 100 (127) | 2–50 (127) | — | — | 96 (177) | 2-N.G. (177) | — | — |
| 75 (207)‖ | 2–37 (207)‖ | — | — | 60 (180) | 2–32 (180) | — | — |
| — | — | — | — | 70 (134) | 2–4 (134) | — | — |
| — | — | — | — | 2000 (137) | — | — | — |
| — | — | — | — | 97 (183) | 1–70 (183)<br>1–27 (183) | — | — |
| 66 (184) | 2-N.G. (184) | — | — | — | — | — | — |
| 268 (145) | 4–67 (145) | 264† (146) | 4–63† (146) | 200 (209) | — | — | — |

## TABLE AIII

*The $k_{cat}$ values and the enzyme concentrations given were calculated from the data in Tables I and II as described in the text. The $k_{cat}$ values are expressed in units of $min^{-1}$ and the enzyme concentrations as $\mu M$. If the molecular weight or number of subunits was not available for a particular source, a value from a closely related organism was used. These cases are indicated by footnote*

| | *E. coli* | | Yeast | | *D. discoideum* | |
|---|---|---|---|---|---|---|
| Enzyme | $k_{cat}$ | [E] | $k_{cat}$ | [E] | $k_{cat}$ | [E] |
| Glycogen synthetase | 35 300 | 3 | 27 300 | 0·65 | 66 | 3·3§ |
| | 101 000 | 2 | | | | |
| Glycogen phosphorylase | — | — | 33 750 | 2·5 | 1 500 | 5·1 |
| | | | 9750 | 17·2 | | |
| UDPG pyrophosphorylase | 21 600†† | 7†† | — | | 40 000 | 4·8§ |
| Glucokinase | — | — | 7600 | 515 | ⟨25·5‖⟩ | 51·8 |
| Hexokinase | — | — | 83 200 | 12·1 | — | — |
| Phosphoglucomutase | 2700† (20) | 64§ | 13 300 | 3·4 | — | — |
| Phosphoglucoisomerase | — | — | 80 300 | 79·5 | 1700‖ | 56·5* |
| Phosphofructokinase | 26 600 | 9·5 | 50 100 | 13·4 | ⟨440‖⟩ | 23·2 |
| | 30 300 | 11·2 | | | | |
| Aldolase | — | — | 12 500† (33) | 210 | — | — |
| Triose P Isomerase | — | — | $1 \times 10^6$† (38) | 23·0 | — | — |
| GA3P dehydrogenase | 5800 | 65§ | 60 000† (42) | 56·0 | — | — |
| 3PGA kinase | 4300 | 38 | 43 500† (45) | 167 | — | — |
| PGA mutase | 6900 | 116§ | 120 600 | 260 | — | — |
| Enolase | 13 200 | 146 | 17 600 | — | — | — |
| Pyruvate kinase | 29 800 | 16·4 | 71 400 | 156 | — | — |
| | 20 900 | 23·4 | | | | |
| Lactate dehydrogenase | 5800 | 1·2 | — | — | — | — |
| Fl,6-bisphosphatase | — | — | 9500 | 2·4 | 2·5‖ | 664·6 |
| G6P dehydrogenase | — | — | 86 800 | 3·5 | — | — |
| 6PG dehydrogenase | 3200 | 5·0 | 4200 | 16·0 | — | — |
| R5P Isomerase | — | — | 12 000 | 173 | — | — |
| Ru5P 3-Epimerase | — | — | 12 000 | 9·1 | — | — |
| Transketolase | — | — | 6800 | 24·7 | — | — |
| Transaldolase | — | — | 4150 | 809 | — | — |
| | | | 2900 | 63·7 | | |
| Rul,5BisPcarboxylase | N.A. | | N.A. | | N.A. | |
| GalIP Uridyltransferase | 16 700 | 24·2 | 59 200† (82) | 0·95 | — | — |
| Galactokinase | — | — | 3300† (83) | 52·3 | — | — |
| Ala transaminase | — | — | — | — | — | — |
| Asp transaminase | 25 800 | 21·9 | 45 200 | 5·0 | — | — |
| Tyr transaminase | — | — | — | — | — | — |
| Glu dehydrogenase (NAD) | — | — | 8050 | 25·0§ | — | — |
| (NADP) | 62 500 | 1·1 | 22 250‖ | 1·9 | | |
| Gln synthetase | 54 000 | 26·1 | — | — | — | — |
| Arginase | — | — | — | — | — | — |
| Glu decarboxylase | 21 000 | 154 | — | — | — | — |
| Ser dehydrase | 10 400 | 8·1§ | — | — | — | — |
| GSSG reductase | 33 600 | 1·2 | 18 000 | 4·7§ | — | — |
| Ornithine decarboxylase | 15 840 | 0·59 | 60 | 3·1 | — | — |

| Rat liver | | Rabbit muscle | | Human RBC | | Spinach leaf | |
|---|---|---|---|---|---|---|---|
| $k_{cat}$ | [E] | $k_{cat}$ | (E) | $k_{cat}$ | [E] | $k_{cat}$ | [E] |
| 9100 | 1·6 | 3000 | 5·2 | — | — | 207 | 4·1 |
| — | — | 15 700† (8) | 102‡ | — | — | 8500 | 0·19 |
| | | | | — | — | — | — |
| — | — | — | — | 55 900 | — | 19 700¶ | 1·1§¶ |
| 4300† (17) | 0·73 | — | — | — | — | — | — |
| — | — | 12 000 | 0·47‡ | — | — | — | — |
| — | — | 73 700 | 28·2 | — | — | — | — |
| — | — | 125 400 | 33·2 | 110 000 | 0·078 | — | — |
| — | — | 61 200 | 42·5 | 57 100 | 0·033 | — | — |
| 2460† (34) | 15·3 | 2133 | 482‡ | 2500 | 0·50 | 1440 | 20·6 |
| | | 2560 | 52·8 | | | | |
| — | — | 350 000 | 96·6 | 573 200 | 1·3 | — | — |
| — | — | 17 400 | 1195 | — | — | 120 000† (43) | 18·2 |
| — | — | 29 210 | 64·4‡ | 30 600 | 1·0 | 32 300 | 11·6 |
| | | 45 000 | 115 | | | | |
| — | — | 57, -64 000 | 173 | — | — | — | — |
| 7900 | 25·4 | 11 160 | 135‡ | — | — | — | — |
| | | 8000 | 292 | | | | |
| 108, -114 400 | 16·4-17·4 | 95 000 | 54·7‡ | 82 500 | 0·069 | — | — |
| | | 72, -81 600 | 65-73·1 | | | | |
| — | — | — | — | — | — | — | — |
| 4800-6000 | 29-36·5 | 3200 | 19·5 | — | — | 8100 | 1·1 |
| | | | | | | 17 400 | 0·66 |
| 27 300 | 4·4 | — | — | (11 400†)(67) | 0·035 | | |
| — | — | — | — | 1560† (70) | 0·40 | — | — |
| — | — | — | — | — | — | 121 000† (72) | 0·37 |
| — | — | — | — | — | — | — | — |
| 200 | 25·0 | — | — | (567) | 0·22 | — | — |
| — | — | — | — | — | — | — | — |
| N.A. | | N.A. | | N.A. | | 1280 | 124 |
| — | — | — | — | — | — | — | — |
| — | — | — | — | 4·4 | 1·4 | — | — |
| 57 100 | | — | — | — | — | — | — |
| 14 500 | 35·4 | — | — | — | — | — | — |
| 30 700 | 21·6 | — | — | — | — | — | — |
| — | — | — | — | — | — | — | — |
| 3120-3560 | 21·1-24·1 | 381-436 | 4·8-5·4‡ | — | — | — | — |
| 626 600 | 12·0 | — | — | — | — | — | — |
| — | — | — | — | — | — | — | — |
| 16, -18 900 | 8·9-10·1 | — | — | — | — | — | — |
| 27 000† (100) | 1·6 | — | — | 19 000 | 0·058 | — | — |
| 2100 | 0·015 | — | — | — | — | — | — |

TABLE AIII—continued

| Enzyme | *E. coli* $k_{cat}$ | *E. coli* [E] | Yeast $k_{cat}$ | Yeast [E] | *D. discoideum* $k_{cat}$ | *D. discoideum* [E] |
|---|---|---|---|---|---|---|
| Arg decarboxylase | 4900 | 1·9 | — | — | — | — |
| DAP decarboxylase | 2000† (106) | — | N.A. | | N.A. | |
| DHPA reductase | 30 000† (107) | — | N.A. | | N.A. | |
| Asp transcarbamoylase | 100 000† (108) | 84·6 | — | — | — | — |
| CarbamoylP synthetase | 980 | 4·8 | — | — | — | — |
| Aspartase | 13 100 | 78·9 | — | — | — | — |
| Lys decarboxylase | 800 000 | 67·6 | — | — | — | — |
| 3PGA dehydrogenase | 1100 | 15·4 | — | — | — | — |
| UDPG 4-epimerase | — | — | 3900† (82) | 28·7 | — | — |
| Glycerol kinase | 11 600† (114) | 97·2 | — | — | — | — |
| Uridine phosphorylase | — | — | — | — | 803‖ | 7·8§ |
| PEP carboxylase | 35 400 | 7·4 | — | — | — | — |
| Pyruvate carboxylase | — | — | ⟨3900‖⟩ | 5·0 | — | — |
| PDC | 105 800 | 27·2 | 232 000 | 15·4 | 7440‖ | 5·2§ |
| Citrate synthase | 42 000 | — | 17 600‖ | 11·8§ | 12 200 | 39·3§ |
| Isocitrate dehydrogenase | 11 900 | 47·4 | 10 680 | 7·6 | 810‖ | 20·1§ |
| SuccinylCoA synthetase | 4700 | 108 | — | — | — | — |
| alpha-KGDC | 3750 | 22·6 | — | — | 8225‖ | 5·1§ |
| Succinate dehydrogenase | 121 | 89·3 | — | — | — | — |
| Malate dehydrogenase | 33 000 | 91·0 | — | — | 38 500 | 12·5§ |
| Malic enzyme | 35 400 | 2·6 | — | — | 172‖ | 44·6§ |

† Given in the reference.
‡ rat muscle.
§ Assumed number of subunits.
‖ Assumed molecular weight.
¶ porcine liver.
†† bovine heart.
ADPG pyrophosphate.
⟨⟩ $k_{cat}$ for subunit.

## TABLE AIV

*Intracellular metabolite concentration. Metabolite concentrations are given in μM*

| Substrate | *E. coli* | Yeast | *D. discoideum* | Rat liver |
|---|---|---|---|---|
| AcCoA | 350 (185) | — | 12 (186) | 39 (187) |
| ADP | 823 (189) | 320-1300 (190, 191) | 200 (192) | 1700 (187) |
| ADPG | — | — | — | — |
| AMP | 151 (189) | 170-300 (191) | — | — |
| ATP | 2641 (189) | 1100-1900 (191, 190) | 700 (192) | 3535 (187) |
| Ala | — | 7, −25 000 (191) | 970 (186) | 1255-1717 (187) |
| Arg | — | 18 000 (191) | — | — |
| Asp | 433-1400 (189, 185) | 3, −13 000 (191) | 370 (186) | 1068-1717 (187) |
| CoA | — | — | — | 180-195 (187) |
| Carbamoyl P | — | — | — | — |
| Citrate | 12 990 (189) | 700 (191) | 60 (186) | 375 (187) |
| Citrulline | — | 5000 (191) | — | — |
| DAP | — | — | — | — |

| Rat liver | | Rabbit muscle | | Human RBC | | Spinach leaf | |
|---|---|---|---|---|---|---|---|
| $k_{cat}$ | [E] | $k_{cat}$ | [E] | $k_{cat}$ | [E] | $k_{cat}$ | [E] |
| — | — | — | — | — | — | — | — |
| N.A. | | N.A. | | N.A. | | N.A. | |
| N.A. | | N.A. | | N.A. | | N.A. | |
| — | — | — | — | — | — | — | — |
| 8100 | 34·8 | — | — | — | — | — | — |
| — | — | — | — | — | — | — | — |
| — | — | — | — | — | — | — | — |
| — | — | — | — | — | — | — | — |
| — | — | — | — | — | — | — | — |
| — | — | — | — | — | — | — | — |
| 247 | 10·1 | — | — | — | — | — | — |
| — | — | — | — | — | — | 11 110 | 0·62 |
| (3800†) (120) | 7·4 | — | — | — | — | — | — |

| Rat liver | | Muscle | | Pig heart | | Plant | |
|---|---|---|---|---|---|---|---|
| $k_{cat}$ | [E] | $k_{cat}$ | [E] | $k_{cat}$ | [E] | $k_{cat}$ | [E] |
| — | — | — | — | 308 | 47·3 | — | — |
| 13 000† (127) | 4·3 | — | — | 17 000† (177) | 10·7 | — | — |
| 3400¶ | 14·6¶ | — | — | 1900 | 109 | — | — |
| — | — | — | — | 8400 | 17·3 | | |
| — | — | — | — | 840 | 11·1§ | — | — |
| — | — | — | — | (10 000†) (139) | 15·6 | — | — |
| 2700 | 18·5 | — | — | 7400 | 154 | — | — |
| 8040 | 24·9 | 5280 | 1·2‡ | 2060†† | 13·1§†† | — | — |

| Rat muscle | Human RBC | Mung bean seedling | Rat heart | Rabbit RBC |
|---|---|---|---|---|
| 1.3 (187) | — | — | 9·6 (188) | — |
| 1059 (187) | 126 (193) | — | 876–1292 (194, 187) | 500 (195) |
| — | — | 200† (211) | — | — |
| — | 50 (193) | 10·9 (196) | 123 (194) | 60 (195) |
| 3075 (187) | 1130 (193) | 10·9 (196) | 1000–5600 (197, 187) | 1700 (195) |
| — | — | — | 996–2453 (198, 188) | — |
| — | — | — | 394 (198) | — |
| — | — | — | 1340–3504 (188, 198) | — |
| 1.7 (sheep) (187) | — | — | 43–80 (199, 187) | — |
| — | — | — | — | — |
| — | — | — | 70–387 (197, 187) | 138 (rat) (187) |

TABLE AIV—continued

| Substrate | *E. coli* | Yeast | *D. discoideum* | Rat liver |
|---|---|---|---|---|
| DHPA | — | — | — | — |
| DHAP | 203 (189) | 330 (190) | 100 (200) | 40-50 (187) |
| E4P | — | — | — | — |
| F6P | — | 650 (190) | 71 (64) | 75-100 (187) |
| F1,6-bisphosphate | 1900 (185) | 1700-4500 (190, 191) | 50 (64) | 23-39 (187) |
| Fumarate | — | — | 30 (186) | 108 (187) |
| GTP | 700 (185) | — | — | — |
| Gal | — | — | — | — |
| Gal 1P | — | — | — | — |
| Glucose | — | — | 500 (192) | 9860-10 200 (187) |
| G 1P | — | ≤100 (191) | 20 (192) | 16 (187) |
| G 6P | 801 (189) | 2300 (190) | 216 (64) | 231-284 (187) |
| Glu | 17 363 (189) | 15, −35 000 (191) | 1200 (186) | 3480 (187) |
| Gln | — | 15, −35 000 (191) | — | 5110-7260 (187) |
| GSSG | — | — | — | — |
| GA3P | — | 400-1200 (201) | 10 | 16 (187) |
| Glycerol3P | 195 (189) | — | — | 202-1010 (187) |
| Glycogen | — | — | 3420‡ (192) | 36 700‡ (187) |
| Isocitrate | — | — | — | 29 (187) |
| alpha-KG | 476 (189) | 200-5000 (191, 190) | 10 (186) | 202 (187) |
| Lactate | — | — | — | 2340 (187) |
| Leu | — | — | — | — |
| Lys | — | — | — | — |
| Malate | 900-1559 (185, 189) | — | 208 (186) | 491 (187) |
| NAD | 1256-2078 (210) | 1000-1600 (191) | 25 (204) | 1097 (187) |
| NADP | — | 20-150 (191) | 25 (204) | 97 (187) |
| NADPH | — | 50-150 (191) | 30 (204) | 433 (187) |
| $NH_3$ | — | 30 000 (191) | — | 678 (187) |
| OAA | — | <50 (191) | 1 (186) | 10 (187) |
| Ornithine | — | 7000 (191) | — | — |
| Pi | — | 22 000 (190) | 3000-30 000 (192) | 4863-5671 (187, 205) |
| PEP | 91-300 (189, 185) | <30 (190) | — | 143 (187) |
| 6PG | — | 100-300 (191) | 18 (64) | 27 (187) |
| 2PGA | — | 420-1100 (201) | — | 49 (187) |
| 3PGA | — | 100-260 (201) | — | 410 (187) |
| $PP_i$ | — | — | — | 14-22 (187) |
| Pyruvate | 390 (189) | 1600 (190) | 60 (186) | 187 (187) |
| R 5P | — | — | 26 (64) | — |
| Ru 5P | — | — | 24 (64) | — |
| Ser | — | — | — | 3708 (rabbit) (205) |
| Succinate | — | — | 1670 (186) | 1068 (187) |
| Tyr | — | — | — | — |
| UDPG | 1299 (189) | 300 (191) | 330 (192) | 330 (187) |
| Uridine | — | — | — | — |
| Xyu 5P | — | — | 14 (64) | — |

† Spinach.

‡ Site concentration, assuming average of one site/12 glucose units.

N.D. = Not detected.

| Rat muscle | Human RBC | Mung bean seedling | Rat heart | Rabbit RBC |
|---|---|---|---|---|
| — | — | — | — | — |
| 40–46 (187) | 12 (193) | 0·7 (196) | 12 (201) | 10 (195) |
| — | — | 1·8 (196) | — | — |
| 362 (187) | 11 (193) | 8·4 (196) | 19 (197) | 11 (195) |
| 46 (187) | 5 (193) | 1 (196) | 8·5 (197) | 7 (195) |
| — | — | — | 105 (188) | — |
| — | — | — | — | 230 (195) |
| — | — | — | — | N.D. (195) |
| — | — | — | — | — |
| 2377 (187) | — | — | 762 (187) | 6170 (195) |
| 65 (187) | — | — | 36 (frog) (187) | 6 (195) |
| 1033 (187) | 27 (193) | 36 (196) | 136 (201) | 62 (195) |
| 2067 (187) | — | — | 4525–6132 (188, 202) | — |
| — | — | — | 4786 (198) | — |
| — | — | — | — | 5·6 (195) |
| 21 (203) | 4 (193) | 0·6 (196) | 3 (302) | 3 (195) |
| 168 (187) | — | — | 78 (187) | — |
| 1500† (187) | — | — | 633† (197) | — |
| — | — | — | 34 (187) | — |
| 78 (187) | — | — | 70–143 (202, 188) | — |
| 2660–6700 (187) | — | — | 4790 (188) | 3810 (195) |
| — | — | — | — | — |
| — | — | — | 721 (198) | — |
| 129 (187) | — | — | 180–400 (20, 188) | — |
| 310 (187) | — | — | 426 (187) | — |
| — | — | — | 5·8 (187) | 22 (195) |
| — | — | — | 120 (187) | — |
| 349 (187) | — | — | — | — |
| 26 (187) | — | — | — | — |
| — | — | — | — | — |
| 5500 (187) | — | — | 4250 (187) | 50 (195) |
| | | | | |
| 8 (187) | 12 (193) | — | 4 (201) | 10 (195) |
| — | — | 0·4 (196) | — | 7·5 (195) |
| 5 (203) | 7 (193) | — | 3 (201) | 5 (195) |
| 40–48 (187) | 48 (193) | — | 26 (201) | 46 (195) |
| — | — | — | — | N.D. (195) |
| 56 (187) | — | — | 39–260 (187, 188) | 90 (195) |
| — | — | 4·4 (196) | — | trace (195) |
| — | — | 0·7 (196) | — | 120 (195) |
| — | — | — | 344 (198) | — |
| | | | | |
| — | — | — | 496 (188) | — |
| — | — | — | — | — |
| 43 (187) | — | — | — | 50 (195) |
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