γM preparations were purified by different methods.

Although it is not unreasonable that the γM molecule might have an ultrastructure somewhat resembling that of the αM globulin, since many of their physicochemical properties indicate a strong similarity, we consider it unlikely that the spider-like particles observed in the γM preparations represented a contamination with αM globulin molecules for the following reasons. (i) There were recognizable differences in the shape of these two structures, the spider-like structures in the γM preparations being more slender and less symmetric with longer, more flexible side legs than the αM particles. (ii) No contamination with αM globulins was detectable in the γM preparations by the Ouchterlony technique or immunoelectrophoresis. The maximum contamination that could escape detection was about 12 to 15 μg/ml, which would amount to about 2 percent of the total protein in the γM preparations. Therefore, the number of αM molecules expected in the preparations would be far less than the observed number of spider-like particles. (iii) In the γM preparations, structures could be seen at various stages of transition from apparently intact extended spider-like particles to those whose legs folded together to form small loops, which were remarkably similar to the loops surrounding the poliovirus capsids in the poliovirus-γM complexes (*4*).

In Höglund and Levin's (*1*) and Höglund's (*2*, *12a*) electron-microscopic studies of normal human γM globulin and γM antibodies against T2 bacteriophage, the γM molecule was reported to have an ovoid shape with dimensions of about 200 × 300 Å. This large ellipsoid structure is not in accordance with our observations on both pathological and normal human γM globulins. In addition, Rowe (14) has pointed out that the molecular weight, calculated on the basis of Höglund and Levin's dimensions differs grossly from values generally accepted for γM.

Humphrey and Dourmashkin (*13*) and Feinstein and Munn (*14*) have published electron micrographs showing filamentous structures, considered to represent γM antibodies, attached to the erythrocyte membrane and bacterial flagella, respectively. The length of these structures, which appears to be about 100 to 150 Å, is compatible with the size of the isolated folded γM molecules that we examined. The electron microscopic appearance of complexes of virus and γM antibody has been studied in detail (*3*, *4*). The flexibility of the γM molecule and the tendency to react with the virus with the formation of two to four loops on the surface of the virus capsid are consistent with our present results. The structure here considered to represent the γM molecule is also compatible with the suggestion (*15*) that the molecule should have five 6*S* to 7*S* subunits (Fig. 1a).

That γM globulins apparently yield intrinsic viscosity values which are higher than those typical for "globular" proteins (*16*) has been attributed to a structure that is either a rigid asymmetric ellipsoid or one that has a high degree of intramolecular, rotational freedom. The latter explanation is compatible both with data from measurements of fluorescence depolarization (*17*) and with the observation that the γM subunits are probably linked by only two disulfide bonds per subunit (*18*). This concept of great intramolecular flexibility was supported by our ultrastructural study.

Our data are also compatible with earlier data on fluorescence depolarization (*19*), an indication that the $\alpha_2$M molecule is more rigid than the γM molecule. The ellipsoidal or spherical structure proposed earlier (*1*, *20*) for the $\alpha_2$M globulin is difficult to reconcile with the electron-microscopic appearance of this molecule in our study.

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## Enzyme Concentrations in Tissues

Abstract. *Apparent enzyme concentrations in cells and in mitochondria are calculated from data available in the literature. These values are $10^{-6}$ to $10^{-5}$ moles per kilogram of tissue. It is pointed out that these concentrations are much higher than those used in enzymatic studies in vitro. Metabolic interpretations of experiments in vitro should consider this additional departure from conditions in vivo.*

Experiments with purified enzymes for the study of metabolic control attempt to use physiological concentrations of substrates and effectors. Because in most cases sensitive assays are usually available for such studies, enzyme concentrations of about $10^{-7}$ to $10^{-10}$ mole/liter are used. In addition to limitations of technique and insufficient quantities of pure enzyme, a lack of knowledge of cellular enzyme concentrations has prevented adequate study of the effects of enzyme concentrations on proposed metabolic regulatory interactions in vivo.

Quite different kinetic behavior results when high concentrations of enzymes ($10^{-5}$ to $10^{-6}$ mole/liter) are used instead of assay concentrations ($10^{-7}$ to $10^{-10}$ mole/liter). I have known (*1*) that $10^{-5}M$ palmityl coenzyme A, a proposed regulator for citrate synthase, is effective against $10^{-8}M$ enzyme but not inhibitory for $10^{-6}M$ enzyme. Frieden and Colman (*2*) showed that $10^{-6}M$ glutamate dehydrogenase ex-

Table 1. Apparent concentrations of some enzymes. S.M., skeletal muscle.

| Enzyme | Tissue | Concentration ($10^{-5}$ mole/kg of tissue) |
|---|---|---|
| Phosphoglucomutase | Rabbit S.M. | 0.55 |
| Aldolase | Rabbit S.M. | 4.5 |
| Aldolase | Rat S.M. | 4.5 |
| Aldolase | Rat liver, heart, brain | 0.3 |
| $\alpha$-Glycerophosphate dehydrogenase | Rabbit muscle | 0.4 |
| Citrate synthase | Heart* | 0.8–1.2 |
| Citrate synthase | Liver* | 0.03–0.13 |
| Citrate synthase | Kidney* | 0.3 |

* From pig, dog, rat, and pigeon.

hibits allosteric behavior toward guanosine triphosphate but that no sigmoidal kinetics can be seen at $10^{-9}M$ glutamate dehydrogenase, the usual assay concentration. The dissociation of this enzyme and others at low concentration is well known.

I now report my calculations of the apparent concentration (mole per kilogram of tissue) of a few enzymes in cells. This concentration is surprisingly high in relation to concentrations of substrates and of cofactors (*3*). For an order-of-magnitude calculation, one can start with the approximation that most soft tissues in animals are about 20 percent protein ($p$). If such tissues ($t$) contain about 1000 different enzymes ($e$) of an average molecular weight of $1 \times 10^5$, then the average apparent concentration is:

$$\frac{200\ g_p/kg_t}{(10^5\ g/mole)\,(1000)} = 2 \times 10^{-6}\ mole_e/kg_t$$

For some enzymes whose molecular weight and turnover numbers are available, the calculation can be made as follows:

$$\frac{U_e/g_e}{U_e/kg_t} = g_e/kg_t$$

$$\frac{g_e/kg_t}{e\ (mol\ wt)} = mole_e/kg_t$$

For citrate synthase in pig heart (*4*), the calculation would be:

$$\frac{39{,}000\ U_e/kg_t}{(33{,}000\ U_e/g_e)\,(90{,}000\ g/mole)} = 1.3 \times 10^{-5}\ mole_e/kg_t$$

The apparent concentrations of a number of enzymes calculated in this way assume the enzymes to be located throughout the cell (Table 1). For mitochondrial enzymes, the apparent concentration would be five times the calculated cellular concentration, because in most cells mitochondria occupy 20 percent of the volume of the whole cell.

Bachmann *et al.* (*5*) determined the activity of a number of Krebs-cycle enzymes in pig heart mitochondria. With these data it is possible to calculate apparent concentrations of a number of mitochondrial enzymes. Their data are given as enzyme units (converted to moles of enzyme) per milligram of mitochondrial protein. If one assumes that the protein content of mitochondria ($m$) is about 20 percent, then 1 mg of mitochondrial protein ($mp$) represents $5 \times 10^{-6}$ kg of mitochondria. With citrate synthase as an example again and using the data of Bachmann *et al.* (*5*), we find that:

$$\frac{1.3 \times 10^{-10}\ mole/mg_{mp}}{5 \times 10^{-6}\ kg_m/mg_{mp}} = 2.6 \times 10^{-5}\ mole/kg_m$$

Table 2 shows the concentration for several pig heart enzymes calculated with both methods, and a fair agreement is seen between the two calculations. Evidence indicates that these enzymes are located exclusively in outer membranes of the mitochondria (*5*); hence, these figures must be increased by a factor of 8 to 10 to obtain apparent concentrations of the enzymes in the outer membrane.

If one considers that the volume of an average mitochondrion is $1.0 \times 10^{-15}$ liter, then just one enzyme molecule per mitochondrion would represent an apparent concentration of $1.5 \times 10^{-9}$ mole/kg of mitochondria. Similar concentrations would exist for one enzyme molecule per bacterial cell.

The concentrations of metabolic intermediates in rat heart (*6*) and in mouse brain (*7*) are in the range of $4 \times 10^{-4}$ to $1 \times 10^{-6}$ mole/liter. In rat liver (*4*), the citrate synthase is $0.9 \times 10^{-6}$ mole per kilogram of tissue ($4.5 \times 10^{-6}$ mole per kilogram of mitochondria), while its substrate concentrations (*8*) are (mole/liter): oxaloacetate, $4.4 \times 10^{-6}$; acetyl coenzyme A, $2.6 \times 10^{-5}$; citrate, $1 \times 10^{-4}$; and coenzyme A, $4.6 \times 10^{-5}$. In rat heart (*4*), the citrate synthase is $1.3 \times 10^{-5}$ mole per kilogram of tissue ($6.5 \times 10^{-5}$ mole per kilogram of mitochondria), whereas the concentrations are (mole/liter): oxaloacetate, $1 \times 10^{-6}$ (*11*) [$\sim 10^{-8}$ mole/liter in mitochondria (*9*)]; acetyl coenzyme A, $2 \times 10^{-6}$ (*11*); and citrate, $1.2 \times 10^{-4}$ (*11*).

Although these are rough calculations with a number of objections, both theoretical and practical, the following points seem to be valid. (i) Enzyme concentrations in cells are orders of magnitude higher than those concentrations usually used in vitro to study them. (ii) Enzyme concentrations in cells are sufficiently high so that, for usual binding constants, significant fractions of some substrates may be protein-bound. (iii) At high enzyme concentrations, regulatory behavior may appear or disappear in relation to that observed at assay concentrations. These considerations add another cautionary note concerning the translation of data on purified enzymes in vitro into corresponding metabolic terms.

Table 2. Apparent concentrations of enzymes in pig heart mitochondria ($10^{-5}$ mole/kg).

| Enzyme | Mitochondria | |
|---|---|---|
| | From total tissue content | From Bachmann *et al.* (*5*) |
| Citrate synthase | 6.5 (*4*) | 2.6 |
| Isocitrate dehydrogenase | | 9.2 |
| $\alpha$-Ketoglutarate dehydrogenase | | 0.6 |
| Fumarase | 0.25 (*3*, *10*) | 0.8 |
| Malate dehydrogenase | 3.0 (*11*) | 5.0 |
| Lipoyl dehydrogenase | 1.0 (*10*) | |
| Cytochrome *a* | 16 (*12*) | 22 |

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