Regulation of Protein Synthesis in Heart Muscle

I. EFFECT OF AMINO ACID LEVELS ON PROTEIN SYNTHESIS*

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SUMMARY

Protein synthesis in heart muscle has been studied in the isolated rat heart perfused with buffer containing glucose and various amino acid mixtures. The rate of synthesis was evaluated by measuring incorporation of 14C-phenylalanine into whole heart protein and myosin. Protein degradation was estimated by measuring the fall in the specific activity of the free amino acid pool and the release of phenylalanine from the heart. 14C-Phenylalanine was suitable for these studies since it equilibrated rapidly with an intracellular pool of stable size and was not converted to other amino acids. Participation of the intracellular free amino acid pool as an intermediate in the pathways of protein synthesis was established. Synthesis of whole heart protein and myosin was increased about 40% when amino acid levels were increased from 1 to 5 times normal plasma levels. A group of 12 amino acids substituted for the complete mixture in producing this effect. Protein degradation appeared to occur at about twice the rate of protein synthesis during perfusion with normal plasma levels of amino acid. When perfusate levels of amino acid were raised, cellular levels of 12 amino acids increased. In general, intracellular amino acid levels reached stable values within 30 min. These studies indicated that protein synthesis in heart muscle could be stimulated by raising perfusate amino acid levels above those normally found in rat plasma. Although net uptake of isoleucine and leucine was observed, suggesting that these amino acids were used as oxidative substrate, the increased rate of synthesis appeared to be due to a direct effect on the pathway of protein synthesis rather than an indirect effect mediated by changes in energy levels of the cell.

Experimental Procedure

Perfusion of Hearts for Estimation of Amino Acid Levels and Protein Synthesis—Hearts were perfused by a modified Langendorff method as described earlier (18). A preliminary perfusion (10 min) was carried out using Krebs-Henseleit bicarbonate buffer (19) gassed with O2 : CO2 (95:5%) and containing heparin (1 mg per ml), 14C-amino acids, and glucose (15 mM). This buffer passed through the heart a single time and was discarded. Recirculation of a measured volume of buffer containing 14C-amino acids, other 14C-amino acids, and glucose followed the preliminary perfusion. Of the radioactive buffer, 10 ml usually were washed through the heart to reduce dilution of the specific activity of 14C-amino acid. Recirculation was con...
Changes in perfusate radioactivity were determined by counting aliquots in a liquid scintillation spectrometer. Uptake and release of free amino acids by the heart were estimated by measuring changes in perfusate levels by ion exchange chromatography (20) using a model 120C Beckman amino acid analyzer. Samples of perfusate were prepared for amino acid analysis by precipitation of protein with 1% picric acid containing norleucine as internal standard (21). Picric acid was removed by passage of the supernatant through a column (1.5 × 10 cm) of Dowex 1-Cl and the amino acids were eluted from the column with 0.1 N HCl. The eluate was lyophilized and the residue dissolved in 10 ml of H₂O. Of this solution, 2.5 ml were added to 0.3 ml of 10 N HCl and the solution was heated in a boiling water bath for 2 hours. Both the hydrolyzed and unhydrolyzed extracts were again lyophilized and the residue dissolved in citric acid buffer, pH 2.2 (22), for application to the amino acid analyzer columns. Specific activity of perfusate phenylalanine was estimated by collecting a portion of the eluate (20%) from the column of the analyzer and counting in a liquid scintillation spectrometer. Specific activity was calculated using the following formula:

\[
\text{specific activity of phenylalanine (dpm/mole)} = \frac{100 \times \text{average radioactivity of phenylalanine peak (cpm/ml)}}{\text{volume of phenylalanine peak (ml)}}
\]

At the end of perfusion, hearts to be used for estimation of amino acid content or 14C-amino acid incorporation were cut into a beaker of 0.15 N NaCl that was sitting in an ice bath. The heart was opened, rinsed, and blotted on filter paper. Hearts were frozen by clamping between blocks of aluminum cooled to the temperature of liquid nitrogen (23) and weighed while frozen. They were then powdered in a percussion mortar at liquid nitrogen temperature (24). Amino acids were extracted from the heart powder with picric acid and heart amino acid content and 14C-phenylalanine specific activity were determined as described above. Intracellular levels of various amino acids were calculated using the following formula:

\[
\text{Heart content (moleals/100 g)} = \text{per fusate concentration (moleals/ml)} - \text{Sorbitol space (ml/100 g)}
\]

\[
\text{total water (ml/100 g)} - \text{sorbitol space (ml/100 g)}
\]

Another sample of heart powder (approximately 0.25 g) was extracted with 5 ml of 0.5 N perchloric acid. The extract was made alkaline by addition of concentrated NH₄OH and the radioactivity determined. 14C-Amino acid space was calculated from these measurements by the following formula:

\[
\text{14C-amino acid space (ml/g)} = \frac{(\text{heart powder (g)} \times \text{total water (ml/g)} + 5 \text{ml})}{\text{radioactivity of heart extract (dpm/ml)}}
\]

\[
\text{weight of heart powder (g) \times radioactivity of perfusate (dpm/ml)}
\]

The precipitated protein, suspended in 0.5 N perchloric acid containing 1 mmol 14C-phenylalanine, isoleucine, glycine, or lysine, was heated for 15 min in a boiling water bath and washed twice with the same solution. The protein was next washed with 0.5 N perchloric acid followed by ethanol-chloroform-ether (3:2:1), and finally by ether (25). The purified protein was dissolved in concentrated formic acid and plated on weighed stainless steel planchets. After reweighing the planchets, radioactivity was determined in a gas flow counter. Correction for self-absorption was made. In several experiments, the micromoles of phenylalanine incorporated into heart protein were calculated using the following formula:

\[
\text{Phenylalanine incorporation (\mu moles/g protein)} = \frac{\text{incorporation (dpm/mg)} \times 1000}{\text{specific radioactivity of heart phenylalanine (dpm/\\mu mole)}}
\]

**Perfusion of Hearts for Estimation of Extracellular Space and Total Water Content**—Hearts were removed and perfusion was begun as described above. Buffer used during the recirculation period contained 14C-D-sorbitol (2.5 mm), 14C-amino acids at the concentrations normally found in rat serum, and glucose (15 mm). At the end of perfusion, hearts were rinsed in 0.15 N NaCl, blotted, and frozen. 14C-Sorbitol space was determined as described above. Total water was estimated by drying a sample of heart powder to constant weight in a vacuum desiccator (26).

**Perfusion of Hearts for Estimation of ATP, Creatine, and Glycogen**—Buffer used during the recirculation period contained glucose (15 mm) and 14C-amino acids at the concentrations indicated in Table X. Hearts were frozen with a Wollenberger clamp (29) while still being perfused. For estimation of ATP and creatine-P, heart powder (approximately 1 g) was extracted with 1 N perchloric acid. The extract was neutralized with KOH and assayed by enzymatic methods involving glucose 6-phosphate dehydrogenase, hexokinase, and creatine kinase (27). Glycogen was determined on samples of heart powder as described earlier (24).

**Preparation and Amino Acid Composition of Heart Myosin**—Samples of heart powder (2 to 3 g) were used for preparation of myosin by the procedure of Weeds and Hartley (28). When working with small amounts of protein, it was important to determine volumes of myosin pellets and solutions by weighing the tubes and beakers before and after addition of myosin. In this way, toxicity of myosin solutions could be closely controlled. The yield of rat heart myosin was 4 to 6 mg per g. Quantities of myosin were estimated by the absorption at 280 and 260 nm (29) and by the method of Lowry et al. (30). Samples of myosin were prepared for estimation of radioactivity as described above except that trichloroacetic acid was substituted for perchloric acid. Amino acid composition of myosin was estimated after hydrolysis in 6 N HCl at 105° for 24 hours. Nortleucine (1 \mu mole per mg of protein) was added as internal standard. Following hydrolysis, the sample was lyophilized and the residue dissolved in citric acid buffer (pH 2.2) for application to the columns of the amino acid analyzer.

**Estimation of Quantity and Radioactivity of Phenylalanine in Heart Protein**—Samples of heart protein that had been prepared for estimation of radioactivity were hydrolyzed in 6 N HCl as described above. Radioactivity in the phenylalanine peak was estimated by collecting the eluate directly from the column of the amino acid analyzer into scintillation vials. One-minute samples were collected in the area of the chromatogram where phenylalanine was expected. A single peak of radioactivity appearing at the time expected for phenylalanine was found. Radioactivity in this peak was determined by summing the disintegrations appearing in each sample. The quantity of phenylalanine in heart protein (micromoles per mg) was determined by the usual amino acid analysis procedure.
Regulation of Protein Synthesis in Heart Muscle. I

RESULTS

Intracellular Amino Acid Pool as Intermediate in Protein Synthesis—Studies of protein synthesis in kidney cortex slices (15) and in skeletal muscle (16) have indicated that amino acid may go directly from the transport system in the cell membrane to protein without mixing with the intracellular pool of free amino acid. Studies in the isolated rat diaphragm (14) have suggested that the intracellular pool of free amino acid was compartmentalized and that not all of these compartments were on the synthetic pathway. Before designing experiments to study regulation of protein synthesis, the involvement of the intracellular pool in protein synthesis was evaluated. If equilibration of the intracellular pool with \(^{14}\)C-amino acid preceded incorporation into protein, a lag in the incorporation of amino acid into protein would be expected. As seen in Fig. 1, a lag in incorporation of \(^{14}\)C-lysine and \(^{14}\)C-glycine was observed during the period when radioactivity of the intracellular water was rising. This lag was more apparent with glycine, for which the radioactivity of the intracellular water increased linearly throughout the experiment. No lag was seen with phenylalanine, for which the radioactivity of intracellular water rapidly reached a plateau. When extracellular \(^{14}\)C-amino acid was washed from the heart after 10 min of recirculation, radioactivity of the intracellular water due to lysine and glycine was well maintained and incorporation continued at the initial rate. Phenylalanine was rapidly lost from the heart during perfusion with buffer free of amino acid and the rate of incorporation fell below that of the first 10 min. After 2 min of washout, radioactivity of the effluent buffer had fallen to less than 1% of that present during the recirculation period. A rapid washout of sorbitol from the extracellular space has been reported (26). These experiments indicated that the intracellular pools of glyline and lysine were on the pathway of protein synthesis. The intracellular pool of phenylalanine equilibrated so rapidly that its role as an intermediate in the synthetic pathway could not be evaluated. Additional experiments to determine the specific activity and size of the \(^{14}\)C-amino acid pool, conversion of the \(^{14}\)C-amino acids into other radioactive products, and the stability of the rate of protein synthesis under these conditions would be required to decide whether the glyline and lysine pools were compartmentalized.

Selection of \(^{14}\)C-Amino Acid for Estimation of Rate of Protein Synthesis—If a radioactive amino acid is to be used to investigate the rate of protein synthesis in cardiac muscle cells, it should have (a) a stable intracellular concentration, (b) a rapid rate of transport or exchange diffusion, or both, (c) no possibility of conversion to other amino acids, and (d) a measurable rate of incorporation.

![Graphs showing intracellular amino acid pool as an intermediate in protein synthesis.](https://via.placeholder.com/150)

Fig. 1. Intracellular amino acid pool as an intermediate in protein synthesis. Hearts were removed and perfused as described under "Experimental Procedure." Preliminary perfusion was performed by using bicarbonate buffer containing glucose (15 mM). Following this perfusion, recirculation of buffer containing either \(^{14}\)C-lysine (0.25 mM), \(^{14}\)C-glycine (0.25 mM), or \(^{14}\)C-phenylalanine (0.08 mM) was begun and continued for 10 or 30 min. In one series of experiments, the \(^{14}\)C-amino acid was washed from the heart after 10 min of recirculation by using bicarbonate buffer containing glucose (15 mM). At the end of perfusion, hearts were cut into saline, blotted, and frozen. Perchloric acid extracts and precipitated protein were prepared for analysis as described. Radioactivity of intracellular water (counts per min per ml) was calculated from the following formula:

\[
\text{Heart content (cpm/100 g)} - \text{perfusate content (cpm/ml perfusate)} = \frac{\text{sorbitol space (ml/100 g)}}{\text{Total water (ml/100 g)} - \text{sorbitol space (ml/100 g)}} 
\]

The solid lines indicate experiments in which buffer containing \(^{14}\)C-amino acid was present throughout. When extracellular amino acid was washed away, the data are indicated by broken lines. Six hearts were perfused for each condition.
into a specific muscle protein. As shown in Fig. 2, \(^{14}\)C-phenylalanine rapidly reached a stable distribution within the heart. This distribution was affected both by the perfusate concentration of phenylalanine and by the concentration of other amino acids. When the intracellular phenylalanine concentration was raised (Table II). In these experiments, incorporation of paper, the concentration was equal to that calculated from the distribution of \(^{14}\)C-phenylalanine. These results indicated that the entire phenylalanine pool rapidly equilibrated. As seen in Table I, the rate of exchange of labeled phenylalanine between the intracellular and extracellular pools was sufficiently rapid to maintain equal specific activities in heart and perfusate phenylalanine. Since these specific activities were equal, any intracellular compartments of phenylalanine also appeared to have reached the specific activity of perfusate phenylalanine. In addition, phenylalanine did not appear to be converted to other amino acids in heart muscle. Phenylalanine hydroxylase has been reported to be lacking in muscle (31) and conversion to other amino acids could not be detected by analysis of acid hydrolysates of heart protein. Of the radioactivity of perfusate or heart extracts, 97% could be recovered in the phenylalanine peak after 90 to 180 min of perfusion. After 3 hours of perfusion, 98% of the radioactivity incorporated into heart protein could be recovered in the phenylalanine peak. These findings, together with measurable rates of incorporation into a specific heart protein, as described later in the paper, indicated that phenylalanine satisfied the criteria for measurements of protein synthesis.

**Effect of Perfusate Amino Acid Concentration on Incorporation of \(^{14}\)C-Amino Acid into Heart Protein—Synthesis of Whole Heart Protein was increased as perfusate amino acid concentration was raised (Table II). In these experiments, incorporation of**

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**TABLE I**

<table>
<thead>
<tr>
<th>Amino acid mixture</th>
<th>Period of perfusion</th>
<th>Specific activity of (^{13})C-phenylalanine</th>
<th>Heart</th>
<th>Perfusate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0.52 f 0.07a</td>
<td>1.97</td>
<td>1.14 f 0.04</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>1026 f 42</td>
<td>180</td>
<td>198 f 0.06</td>
</tr>
</tbody>
</table>

---

**TABLE II**

<table>
<thead>
<tr>
<th>Amino acid mixture</th>
<th>Period of perfusion</th>
<th>(^{13})C-Phenylalanine incorporation into heart protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min</td>
<td>dpm/mg protein</td>
</tr>
<tr>
<td>Only (^{14})C-phenylalanine, 0.08 mm</td>
<td>30</td>
<td>331 f 15a</td>
</tr>
<tr>
<td>All amino acids at normal plasma levels, including (^{14})C-phenylalanine, 0.08</td>
<td>30</td>
<td>358 f 26</td>
</tr>
<tr>
<td>All amino acids at 5 times normal plasma levels, including (^{14})C-phenylalanine, 0.4 mm</td>
<td>30</td>
<td>458 f 42</td>
</tr>
</tbody>
</table>

---

- Mean standard error of the mean.
- \(p < 0.05\) versus only phenylalanine.
- \(p < 0.05\) versus normal plasma levels.
phenylalanine into protein was calculated using the specific activity of heart phenylalanine (Table I). The effect of perfusate amino acid concentration became significant after 90 min of perfusion and was more marked after 3 hours. Since the specific activities of perfusate and heart phenylalanine did not fall significantly when 5 times the normal plasma level was present (Table I), an experimental design was adopted to maintain constant phenylalanine specific activities when normal plasma levels were added (Fig. 3). The volume of perfusate was increased to maintain the same quantity of phenylalanine in the extracellular pool with normal plasma levels as when 5 times the normal plasma level was present. An increase in protein synthesis with higher perfusate concentrations of amino acids was confirmed in these experiments. The effect was particularly apparent after 3 hours of perfusion.

The effect of a wider range of perfusate amino acid concentrations on incorporation of phenylalanine and isoleucine into heart protein indicated that the maximal effect was obtained when 5 times normal plasma levels were added (Table III). The magnitude of the increase of 14C-isoleucine incorporation was comparable to that found with 14C-phenylalanine. The effect of perfusate amino acid concentration was also examined in experiments in which the concentration of phenylalanine was maintained at normal plasma levels (Fig. 4). Incorporation of phenylalanine increased as the concentration of other amino acids was raised from 0 to 2 times normal plasma levels. When 14C-amino acids were added, intracellular phenylalanine concentrations fell and incorporation reached a plateau. The fall in intracellular phenylalanine concentration was presumed to be due to competitive

![Fig. 3](image-url)  
**Fig. 3.** Effect of perfusate amino acid concentration on incorporation of phenylalanine into heart protein. Hearts were perfused as described under "Experimental Procedure" and Fig. 2. When only Phe or all amino acids at normal plasma level were added to the perfusate, 125 ml of buffer were recirculated. When 5 times normal plasma levels of all amino acids were added, 25 ml of buffer were recirculated. Ten milliliters of buffer containing radioactive amino acid were washed through the heart before recirculation was begun. Six to 25 hearts were perfused for each condition indicated. The mean ± S.E. is shown on the figure.

![Fig. 4](image-url)  
**Fig. 4.** Effect of the perfusate concentration of other amino acids on the incorporation of phenylalanine into heart protein. Hearts were perfused as described under "Experimental Procedure." The concentrations of various amino acids used to simulate normal plasma levels are given in Table VII. Per fusate phenylalanine concentration was 0.08 mM in all experiments. Buffer, 125 ml, was recirculated for 3 hours. Six hearts were perfused for each condition. The mean ± S.E. is shown on the figure.

### Table III

*Effect of perfusate amino acid concentration on incorporation of phenylalanine and isoleucine into heart protein*

<table>
<thead>
<tr>
<th>Amino acid mixture</th>
<th>14C-Amino acid</th>
<th>Perfusate radioactivity</th>
<th>14C-Amino acid</th>
<th>14C-Amino acid space</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 min</td>
<td>60 min</td>
<td>cm/µg</td>
</tr>
<tr>
<td>All amino acids at normal plasma level</td>
<td>Phe</td>
<td>122 ± 2*</td>
<td>110 ± 2*</td>
<td>53 ± 2*</td>
</tr>
<tr>
<td></td>
<td>Ile</td>
<td>64 ± 1</td>
<td>55 ± 1</td>
<td>18 ± 1</td>
</tr>
<tr>
<td>All amino acids at 2 times normal plasma levels</td>
<td>Phe</td>
<td>226 ± 2</td>
<td>220 ± 4</td>
<td>63 ± 2</td>
</tr>
<tr>
<td></td>
<td>Ile</td>
<td>123 ± 1</td>
<td>108 ± 1</td>
<td>23 ± 1</td>
</tr>
<tr>
<td>All amino acids at 5 times normal plasma levels</td>
<td>Phe</td>
<td>592 ± 12</td>
<td>572 ± 8</td>
<td>72 ± 3</td>
</tr>
<tr>
<td></td>
<td>Ile</td>
<td>307 ± 6</td>
<td>276 ± 6</td>
<td>31 ± 1</td>
</tr>
<tr>
<td>All amino acids at 10 times normal plasma levels</td>
<td>Phe</td>
<td>1104 ± 12</td>
<td>1132 ± 12</td>
<td>74 ± 3</td>
</tr>
<tr>
<td></td>
<td>Ile</td>
<td>586 ± 4</td>
<td>538 ± 4</td>
<td>31 ± 1</td>
</tr>
</tbody>
</table>

* Mean ± standard error of the mean.
**Table IV**

**Effect of mixture of certain amino acids on incorporation of phenylalanine into heart protein**

Hearts were removed and perfused as described under "Experimental Procedure." Concentrations of various amino acids used to simulate normal plasma levels are given in Table VII. Buffer (25 ml) was recirculated for 3 hours. Radioactive buffer (10 ml) was washed through the heart before recirculation was begun. Six hearts were perfused for each condition.

<table>
<thead>
<tr>
<th>Perfusate amino acid</th>
<th>Times normal plasma level</th>
<th>Phenylalanine incorporation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylalanine</td>
<td>5</td>
<td>2.03 ± 0.06</td>
</tr>
<tr>
<td>Phenylalanine, threonine, serine, valine, methionine, isoleucine, leucine, tyrosine, histidine, proline, tryptophan, cysteine</td>
<td>5</td>
<td>2.12 ± 0.07</td>
</tr>
<tr>
<td>All</td>
<td>5</td>
<td>2.44 ± 0.07</td>
</tr>
</tbody>
</table>

* Mean ± standard error of the mean.

**Table V**

**Effect of perfusate amino acid concentrations on incorporation of 14C-phenylalanine into whole heart protein and myosin**

Hearts were removed and perfused for 3 hours as described under "Experimental Procedure." Perfusate (125 ml) was recirculated when the normal plasma level of phenylalanine was present and 25 ml of perfusate were recirculated when 5 times the normal plasma level was added. Twelve hearts were perfused under each condition and analyzed individually for incorporation into heart protein. Pools of three hearts were used for preparation of myosin. The specific activity of perfusate phenylalanine was 593 X 10^3 dpm per pmole.

ATPase activity of heart myosin averaged 0.87 ± 0.04 pmoles of Pi released per mg per min when assayed at 25°C in a buffer containing 0.25 M KCl, 0.05 M Tris, 5 mM ATP, 5 mM M & CaCl₂, pH 7.5. When CaCl₂ was replaced by MgCl₂, ATPase activity was 0; when CaCl₂ was omitted, 0.14 ± 0.01 pmole of Pi were released per mg per min.

<table>
<thead>
<tr>
<th>Amino acid mixture</th>
<th>14C-Phenylalanine incorporation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heart protein</td>
</tr>
<tr>
<td>Only 14C-phenylalanine, 0.08 mM</td>
<td>1.63 ± 0.00a</td>
</tr>
<tr>
<td>All amino acids at normal plasma levels, including 14C-phenylalanine, 0.08 mM</td>
<td>1.70 ± 0.07</td>
</tr>
<tr>
<td>All amino acids at 5 times normal plasma levels, including 14C-phenylalanine, 0.4 mM</td>
<td>2.36 ± 0.12</td>
</tr>
</tbody>
</table>

* Mean ± standard error of the mean.

**Table VI**

**Comparison of amino acid composition of skeletal and cardiac muscle myosin**

Myosin was prepared as described under "Experimental Procedure." Results are expressed as a ratio of various amino acids to the content of phenylalanine. Phenylalanine content or rat heart myosin was 0.23 ± 0.01 pmole per mg. Amino acid composition of skeletal muscle myosin was taken from the data of Weeds and Hartley (28). Four preparations of rat heart myosin were analyzed.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Skeletal muscle myosin</th>
<th>Rat heart myosin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pmole amino acid/pmole phenylalanine</td>
<td></td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>3.01</td>
<td>2.81 ± 0.05a</td>
</tr>
<tr>
<td>Threonine</td>
<td>1.58</td>
<td>1.48 ± 0.03</td>
</tr>
<tr>
<td>Serine</td>
<td>1.57</td>
<td>1.32 ± 0.06</td>
</tr>
<tr>
<td>Proline</td>
<td>0.78</td>
<td>Not resolved</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>5.95</td>
<td>5.94 ± 0.14</td>
</tr>
<tr>
<td>Glycine</td>
<td>1.41</td>
<td>1.43 ± 0.06</td>
</tr>
<tr>
<td>Alanine</td>
<td>2.68</td>
<td>2.44 ± 0.08</td>
</tr>
<tr>
<td>Valine</td>
<td>1.58</td>
<td>1.48 ± 0.03</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.85</td>
<td>0.42 ± 0.06</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>1.40</td>
<td>1.63 ± 0.02</td>
</tr>
<tr>
<td>Leucine</td>
<td>2.87</td>
<td>2.89 ± 0.04</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.63</td>
<td>0.57 ± 0.05</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Lysine</td>
<td>3.07</td>
<td>2.89 ± 0.09</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.55</td>
<td>0.51 ± 0.01</td>
</tr>
<tr>
<td>Arginine</td>
<td>1.43</td>
<td>1.45 ± 0.06</td>
</tr>
</tbody>
</table>

* Mean ± standard error of the mean.

A group of 12 amino acids was found to have the same effect on phenylalanine incorporation as the complete amino acid mixture (Table IV). Intracellular concentrations of 9 of this group were below 0.35 mM in unperfused heart (Table VII). Intracellular concentrations of proline, cystine, and tryptophan were too low for reliable measurements by the procedures that were employed.

An effect of amino acid concentration on protein synthesis in heart muscle cells was established by measuring the ratio of synthesis of myosin. As shown in Table V, myosin synthesis occurred at about 50% of the rate of whole heart protein. Myosin synthesis was increased approximately 50% when perfusate amino acid levels were raised from 1 to 5 times normal plasma levels. The myosin used for these studies was characterized by possessing (a) a Ca⁺⁺-activated ATPase activity (Table V) and (b) amino acid composition similar to that of skeletal muscle myosin (Table VI).

These results indicated that protein synthesis in heart muscle became dependent upon the intracellular concentrations of several amino acids during perfusion in vitro. The effect could be demonstrated by studying either synthesis of whole heart protein or myosin. The remainder of the experiments in this paper were carried out to describe (a) changes in intracellular amino acid levels when perfusate concentrations were changed, (b) uptake and release of amino acids by the heart, and (c) effects of perfusate amino acid concentrations on levels of high energy phosphates and glycogen in heart muscle.

**Effect of Perfusate Amino Acid Concentrations on Intracellular Concentrations of Various Amino Acids**—When the heart was perfused in vitro with normal plasma levels of amino acid, intracellular levels of other amino acids were added.

inhibition of membrane transport. As shown in Table VII, addition of 5 times normal plasma levels of phenylalanine along with 5 times normal plasma levels of other amino acids resulted in intracellular phenylalanine levels approximately 4-fold greater than shown in Fig. 4. Incorporation of phenylalanine was also 40% greater (Table II). These results suggested that intracellular phenylalanine levels restricted protein synthesis when high perfusate levels of other amino acids were added.
Regulation of Protein Synthesis in Heart Muscle.

Intracellular amino acid concentrations in hearts of normal rats and in hearts perfused for 90 min with buffer containing various mixtures of amino acids

Hearts were removed from anesthesia rats and perfused as described under "Experimental Procedure." Three mixtures of amino acids were employed: (a) phenylalanine alone at the concentration found in normal rat serum, 0.68 mM; (b) all amino acids at concentrations near those normally found in rat serum, and (c) all amino acids at 5 times the concentrations normally found in rat serum. These mixtures are referred to in the table as PHE, 1 X, and 5 X, respectively. In the case of asparagine, glutamine, and arginine, the perfusate levels that were chosen to simulate normal serum levels were significantly different from the serum levels that are reported. This discrepancy arose from the fact that perfusate levels were chosen on the basis of earlier measurements in this laboratory and the values reported by others (21, 32, 33). The serum levels reported in this paper were determined in conjunction with the perfusion experiments. In the case of methionine, the serum concentration (0.05 mM) was added to the perfusate, but only 50% of this value was obtained by analyzing the perfusate medium. Oxidation of methionine may have accounted for this loss. In addition to the amino acids that are reported, tryptophan (0.05 mM) was included in the normal amino acid mixture. Perfusate (25 ml) was recirculated in each case.

In addition to the 90-min values, intracellular concentrations were measured after 30 and 180 min. After 30 min of perfusion, the following concentrations (10 mM) were significantly different from the 90-min value: (a) aspartic acid, 5 X, 348 ± 38; (b) asparagine, 1 X, 121 ± 22; (c) glutamic acid, PHE, 570 ± 43; (d) glutamic acid, 1 X, 784 ± 18; (e) glutamic acid, 5 X, 1106 ± 97; (f) serine, 5 X, 38 ± 5; (g) glycine, PHE, 64 ± 2; (h) glycine, 1 X, 87 ± 8; (i) valine, 5 X, 21 ± 9. After 180 min of perfusion, the following concentrations (10 mM) were significantly different from the 90-min value: (a) aspartic acid, PHE, 51 ± 7; (b) asparagine, 1 X, 77 ± 20; (c) glutamic acid, PHE, 487 ± 1; (d) glutamine, 770 ± 61; (e) leucine, 5 X, 12 ± 4.

Intracellular amino acid concentrations of perfused hearts were calculated assuming that extracellular volume was 22 ml/100 g and the intracellular volume was 56 ml/100 g (33). Extracellular water of perfused hearts was estimated by measuring sorbitol space during periods ranging from 10 to 180 min. Sorbitol space was 56 ml/100 g at all time periods. Total water content of these hearts was 80 ml/100 g at all time periods.

Five pools of six hearts were analyzed to obtain serum and levels in vivo. Three pools of heart and perfusion medium were analyzed to obtain levels in vitro. When only phenylalanine was added to the buffer, eight hearts were included in the pool. When normal serum levels of amino acid were present, six hearts were pooled, and when 5 times normal levels were added, three hearts were pooled. These quantities of heart muscle were needed to obtain reliable analyses.

<table>
<thead>
<tr>
<th>Amino acid analyzed</th>
<th>Extracellular concentration, 10^9 M</th>
<th>Intracellular concentration, 10^9 M</th>
<th>In vivo</th>
<th>In vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum</td>
<td>Added to simulate serum level</td>
<td>PHE</td>
<td>1 X</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>1.4 ± 0.2 M</td>
<td>4.7</td>
<td>90 ± 11</td>
<td>245 ± 50</td>
</tr>
<tr>
<td>Asparagine</td>
<td>6.8 ± 0.5 M</td>
<td>7.4</td>
<td>22 ± 2</td>
<td>50 ± 7</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>12.0 ± 0.8 M</td>
<td>11.6</td>
<td>826 ± 67</td>
<td>1157 ± 89</td>
</tr>
<tr>
<td>Glutamine</td>
<td>65.2 ± 4.1 M</td>
<td>30.5</td>
<td>1085 ± 130</td>
<td>1127 ± 81</td>
</tr>
<tr>
<td>Serine</td>
<td>24.7 ± 3.4 M</td>
<td>17.0</td>
<td>24 ± 3</td>
<td>34 ± 5</td>
</tr>
<tr>
<td>Threonine</td>
<td>20.3 ± 0.06 M</td>
<td>21.8</td>
<td>35 ± 4</td>
<td>51 ± 4</td>
</tr>
<tr>
<td>Glycine</td>
<td>32.6 ± 0.7 M</td>
<td>26.5</td>
<td>62 ± 4</td>
<td>80 ± 4</td>
</tr>
<tr>
<td>Alanine</td>
<td>32.6 ± 0.7 M</td>
<td>1.4</td>
<td>37.7</td>
<td>204 ± 40</td>
</tr>
<tr>
<td>Methionine</td>
<td>5.4 ± 0.1 M</td>
<td>5.0</td>
<td>5.0 ± 0.6</td>
<td>10.9 ± 1.0</td>
</tr>
<tr>
<td>Valine</td>
<td>19.8 ± 0.4 M</td>
<td>19.0</td>
<td>19.0</td>
<td>14.7 ± 3.9</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>10.8 ± 0.4 M</td>
<td>9.1</td>
<td>6.8 ± 0.9</td>
<td>5.8 ± 0.9</td>
</tr>
<tr>
<td>Leucine</td>
<td>16.6 ± 0.6 M</td>
<td>13.1</td>
<td>11.2 ± 1.5</td>
<td>6.5 ± 0.9</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>6.9 ± 0.1 M</td>
<td>7.7</td>
<td>5.9 ± 1</td>
<td>11.5 ± 0.9</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>6.9 ± 0.1 M</td>
<td>6.0</td>
<td>8.0 ± 2</td>
<td>2.9 ± 0.3</td>
</tr>
<tr>
<td>Lysine</td>
<td>38.0 ± 2.1 M</td>
<td>24.2</td>
<td>98 ± 6</td>
<td>161 ± 7</td>
</tr>
<tr>
<td>Histidine</td>
<td>5.2 ± 0.2 M</td>
<td>7.8</td>
<td>19 ± 2</td>
<td>5 ± 0.8</td>
</tr>
<tr>
<td>Arginine</td>
<td>11.9 ± 1.1 M</td>
<td>6.2</td>
<td>24 ± 3</td>
<td>46 ± 5</td>
</tr>
<tr>
<td>Proline</td>
<td>10.8 ± 0.6 M</td>
<td>15.8</td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td>Half-erythine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± standard error of the mean.

Intracellular levels of 11 amino acids increased as perfusate concentrations were raised from 0 to 5 times normal plasma levels (Table VII). Intracellular levels of the branched chain amino acids, glutamine, and lysine were unchanged. Intracellular concentrations of arginine decreased as perfusate levels were raised. Changes in intracellular amino acid levels were rapid. In general, a new steady state was reached within 30 min. However, intracellular levels of aspartic acid, asparagine, and glutamic acid decreased as perfusion was continued, whereas glycine continued to increase. These results indicated that stable intra-
Uptake or release of amino acids was determined using the same hearts employed for analysis of intracellular amino acid concentrations (Table VII). Uptake or release was not detected with other amino acids. Three pools of three to eight hearts were analyzed.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Perfusion concentration, 10^2 mM</th>
<th>Uptake</th>
<th>Release</th>
<th>Efflux rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min of perfusion</td>
<td>90 min of perfusion</td>
<td>180 min of perfusion</td>
<td>0 to 90 min of perfusion</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>9.1 ± 0.2</td>
<td>9.1 ± 0.4</td>
<td>7.2 ± 0.2</td>
<td>0.16 ± 0.02</td>
</tr>
<tr>
<td>Leucine</td>
<td>45 ± 1.0</td>
<td>45 ± 1.3</td>
<td>39 ± 1.6</td>
<td>0.50 ± 0.16</td>
</tr>
<tr>
<td>Glutamine</td>
<td>30.5 ± 3.9</td>
<td>38.9 ± 1.9</td>
<td>47.1 ± 3.6</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>Glycine</td>
<td>26.5 ± 1.4</td>
<td>33.3 ± 1.4</td>
<td>34.7 ± 2.4</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>Alanine</td>
<td>37.7 ± 3.3</td>
<td>54.4 ± 2.8</td>
<td>46.7 ± 2.8</td>
<td>2.8 ± 0.7</td>
</tr>
<tr>
<td>Methionine</td>
<td>178 ± 8</td>
<td>218 ± 8</td>
<td>262 ± 10</td>
<td>6.7 ± 0.7</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>2.4 ± 0.1</td>
<td>3.8 ± 0.2</td>
<td>4.2 ± 0.1</td>
<td>0.23 ± 0.003</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>16.6 ± 0.9</td>
<td>20.5 ± 0.7</td>
<td>19.9 ± 1.0</td>
<td>0.66 ± 0.2</td>
</tr>
<tr>
<td>Arginine</td>
<td>7.7 ± 0.3</td>
<td>8.9 ± 0.3</td>
<td>9.9 ± 0.5</td>
<td>0.20 ± 0.07</td>
</tr>
<tr>
<td>Glycine</td>
<td>6.0 ± 0.3</td>
<td>7.6 ± 0.5</td>
<td>8.3 ± 0.4</td>
<td>0.19 ± 0.04</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>6.2 ± 0.4</td>
<td>7.9 ± 0.1</td>
<td>8.2 ± 0.8</td>
<td>0.28 ± 0.07</td>
</tr>
</tbody>
</table>

- Mean ± standard error of the mean.

Efflux rate was calculated during the first 30 min of perfusion with only phenylalanine added to the perfusate. Buffer (25 ml) was recirculated.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Intracellular amino acid concentration (μmoles/ml)</th>
<th>Perfusion amino acid concentration (μmoles/ml)</th>
<th>Efflux rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>0.33 ± 0.04</td>
<td>253 ± 65</td>
<td>0.037 ± 0.007</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>0.77 ± 0.08</td>
<td>870 ± 43</td>
<td>0.019 ± 0.003</td>
</tr>
<tr>
<td>Glutamine</td>
<td>7.2 ± 0.3</td>
<td>1410 ± 188</td>
<td>0.126 ± 0.024</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.80 ± 0.04</td>
<td>30 ± 5</td>
<td>0.63 ± 0.14</td>
</tr>
<tr>
<td>Serine</td>
<td>0.80 ± 0.05</td>
<td>28 ± 3</td>
<td>0.70 ± 0.15</td>
</tr>
<tr>
<td>Glycine</td>
<td>2.2 ± 0.2</td>
<td>64 ± 2</td>
<td>0.99 ± 0.07</td>
</tr>
<tr>
<td>Alanine</td>
<td>5.9 ± 0.2</td>
<td>239 ± 40</td>
<td>0.64 ± 0.07</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.41 ± 0.02</td>
<td>7.2 ± 0.6</td>
<td>1.65 ± 0.09</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.80 ± 0.08</td>
<td>6.0 ± 1.0</td>
<td>3.08 ± 0.47</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.50 ± 0.13</td>
<td>4 ± 0.5</td>
<td>3.17 ± 0.74</td>
</tr>
<tr>
<td>Lysine</td>
<td>1.1 ± 0.1</td>
<td>137 ± 11</td>
<td>0.20 ± 0.01</td>
</tr>
<tr>
<td>Arginine</td>
<td>0.41 ± 0.05</td>
<td>39 ± 3</td>
<td>0.26 ± 0.02</td>
</tr>
</tbody>
</table>

- Mean ± standard error of the mean.
Effect of amino acid concentration on heart content of ATP, creatine-P, and glycogen

Hearts were perfused as described under "Experimental Procedure." The buffer used for the preliminary perfusion of 10 min contained neither glucose nor amino acids. Buffer used for the recirculation period lasting either 30 or 180 min contained 15 mm glucose in addition to the amino acids listed above. Results are expressed per g of dry heart weight. The number of hearts that were perfused is indicated by figure in parentheses.

<table>
<thead>
<tr>
<th>Amino acid level</th>
<th>Period of perfusion</th>
<th>Tissue content</th>
<th>ATP</th>
<th>Creatine-P</th>
<th>Glycogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal plasma</td>
<td>10 (9)</td>
<td>24.1 ± 1.4</td>
<td>23.3 ± 1.8</td>
<td>120.3 ± 3.9*</td>
<td></td>
</tr>
<tr>
<td>levels</td>
<td>40 (8)</td>
<td>24.0 ± 1.0</td>
<td>19.9 ± 0.9</td>
<td>97.5 ± 4.8</td>
<td></td>
</tr>
<tr>
<td>5 Times normal</td>
<td>190 (7)</td>
<td>20.4 ± 1.4</td>
<td>18.1 ± 1.6</td>
<td>46.7 ± 4.3</td>
<td></td>
</tr>
<tr>
<td>plasma levels</td>
<td>40 (5)</td>
<td>24.1 ± 1.0</td>
<td>21.5 ± 1.0</td>
<td>96.0 ± 6.4</td>
<td></td>
</tr>
<tr>
<td>190 (6)</td>
<td>19.3 ± 1.8</td>
<td>22.3 ± 2.3</td>
<td>96.7 ± 10.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± standard error of the mean.

have been reported to share a common transport system (34). The highest permeabilities were encountered with isoleucine, leucine, and histidine. These amino acids also have been reported to share a common transport system (34, 35). In other experiments in heart muscle, rapid equilibration of leucine and isoleucine across the cell membrane has been observed. Permeabilities of lysine and arginine that share the cationic amino acid transport system (36, 37) were low.

Effect of Perfusate Amino Acid on Heart Content of ATP, Creatine-P, and Glycogen—If higher perfusate levels were increasing protein synthesis by providing alternative oxidative substrates to the heart, higher levels of ATP and creatine-P might result. As seen in Table A, ATP levels fell about 20% during 3 hours of perfusion in vitro with buffer containing normal plasma amino acid levels. This change was not affected by inclusion of higher levels of amino acid in the perfusate. Creatine-P was unchanged as perfusion was continued with either mixture of amino acid. Sparing of glycogen was demonstrable when 5 times normal plasma levels of amino acid were added. This effect was apparent only after 3 hours of perfusion. Sparing of glycogen by the inclusion of fatty substrates in the buffer has been noted in earlier experiments (38). Since branched chain amino acids are metabolized by formation of fatty-CoA derivatives, uptake and oxidation of these compounds may have accounted for glycogen sparing.

**DISCUSSION**

Protein synthesis in muscle is regulated by a variety of factors including insulin (39, 40), growth hormone (1, 3, 41, 42), thyroxine (42), steroids (43, 44), androsterone (45), and the mechanical activity of the tissue (46-48). The mechanisms of these effects are incompletely understood at present. The perfused rat heart appears to be a suitable preparation for study of these problems. The preparation has a stable mechanical performance for periods in excess of 3 hours (18). The level of ventricular pressure development can be closely controlled during perfusion (18). Hormones and substrates reach the cells via the normal capillary bed. The rat heart is sufficiently large to provide adequate material for analysis of free amino acid levels and other metabolites involved in protein synthesis and for preparation of ribosomes and myosin. When compared with the perfused liver, the heart presents a simpler model for studies of mammalian protein synthesis because of slower rates of amino acid oxidation and the absence of conversion of amino acids into glucose. As a result, stable intracellular amino acid levels are much easier to maintain in heart than liver.

Myosin appears to be a suitable protein for investigation of regulation of protein synthesis in cardiac muscle cells. The heart contains approximately 35 mg of myosin per g (49). The yield of purified protein was about 1 to 6 mg per g. Rat heart myosin was found to have a similar amino acid composition to rabbit skeletal muscle myosin. Bánányi et al. (50) found less valine and arginine and more leucine and lysine in myosin of rabbit heart than in myosin of white skeletal muscle. The adenosine triphosphatase activity of rat heart myosin was greater than that reported earlier (51) using myosin prepared from dog heart. We have not determined whether this difference is due to species difference or to the method of preparation. In the present studies, myosin was extracted from hearts that had been pulverized in a percussion mortar at liquid nitrogen temperatures. The preparative procedure was completed within 30 hours of the initial extraction and ATPase activity was assayed immediately.

Synthesis of whole heart protein and myosin can be estimated using the initial rates found during perfusion in vitro. Whole heart protein contains 284 ± 17 μmoles of phenylalanine per g. Since 1.24 μmoles of phenylalanine per g were incorporated in 90 min (Table II), an average of 14.3 days would be required to synthesize the protein in rat heart. Myosin contained 230 μmoles of phenylalanine per g. Since 1.35 μmoles per g were incorporated in 3 hours of perfusion (Table V), 21.3 days would be required to synthesize the myosin found in rat heart. This rate of synthesis may be lower than that found in vivo since synthesis of whole heart protein fell off during this period of perfusion. Myosin has a molecular weight of approximately 500,000 and consists of 2 large subunits with a molecular weight of about 200,000 and 1 or 2 small subunits with a molecular weight of about 20,000 (49). The rate of synthesis estimated in the present experiments was for the whole molecule. In other experiments in which changes in ATPase activity have been measured using myosin prepared from hearts of hypophysectomized rats, ATPase activity was reduced to about 50% of normal 8 days after hypophysectomy (52). This reduction could be due to a change in the small subunits and might suggest that these subunits have a more rapid rate of turnover than the large subunits.

Degradation of heart protein can be estimated from the fall in specific activity of perfusate phenylalanine or from production of phenylalanine by the heart. As discussed earlier, release of phenylalanine into the perfusate suggested that the net rate of protein degradation was 0.7 mg per g per hour. The absolute rate of protein degradation, obtained by the addition of the net rate and the rate of protein synthesis, was about 1.1 mg per g per hour. The fall in specific activity of phenylalanine after 3 hours of perfusion with normal plasma levels of amino acid (Table I) indicated that 0.58 μmoles of phenylalanine had been added for each micromole that was present after 30 min. A total of 2.16 μmoles of free phenylalanine were present in perfusate and heart after 30 min of perfusion. Fall in specific activity of phenylalanine indicated that 2.97 μmoles would have...
been present after 3 hours of perfusion if none had been reincorporated into protein. The absolute rate of protein degradation estimated by dilution of phenylalanine specific activity was 1.2 mg per g per hour, in good agreement with the rate estimated from phenylalanine release. Actually found were 2.57 μmole (Table V11), indicating that 0.4 μmole had been reincorporated.

The net rate of protein degradation estimated by this method was about 0.6 mg per g of heart per hour. Since the heart contains about 160 mg of protein per g, an average of 6 days would be required to deplete heart protein at the rate observed in vitro. Since an average of 14 days was required to synthesize heart protein, whereas only 6 days were necessary for degradation, perfusion in vitro appeared either to have accelerated degradation or to have inhibited protein synthesis. Increased rates of protein degradation have been observed to reduce the rate (53).

The effect of higher perfusate amino acid levels on protein degradation in the heart could not be determined in the present experiments because of an unfavorable ratio between heart size and perfusate volume. During perfusion of the heart with buffer containing glucose, the rate of protein synthesis was increased about 40% when 5 times normal plasma levels of all amino acids were added as compared with normal plasma levels. With either amino acid mixtures, the rate of synthesis decreased during the 2nd and 3rd hours of perfusion to about 60% of the rate observed in the 1st hour. The reduced rates of synthesis observed with these amino acid mixtures were not due to reductions in intracellular amino acid concentrations (Table V11). As shown in the following paper (54), reduced rates of protein synthesis observed in hearts perfused with buffer containing glucose and amino acids were associated with increased numbers of ribosomal subunits and decreased polysomes. When 5 times normal plasma levels of all amino acids were added, smaller numbers of ribosomal subunits and more polysomes were found. A similar effect of amino acid supply on breakdown and reaggregation of liver ribosomes has been found in vitro (55), in the perfused liver (56), and in a cell-free system (57). The effect of amino acid supply on ribosomal aggregation in heart muscle together with comparable ATP and creatine-P levels in hearts perfused with 1 and 5 times normal plasma amino acid levels suggested that higher amino acid levels stimulated protein synthesis directly rather than indirectly by changing the levels of high energy phosphates. The importance of changes in intracellular amino acid levels in accounting for the effects of various factors regulating protein synthesis will be discussed in subsequent papers.

REFERENCES

Regulation of Protein Synthesis in Heart Muscle: I. EFFECT OF AMINO ACID LEVELS ON PROTEIN SYNTHESIS


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