Myocardial Aminoacyl-Transfer-Ribonucleic Acid Synthetase and Aminoacyl-Transferring Enzyme Activity

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The properties of cytoplasmic aminoacyl-tRNA synthetase and aminoacyl-transferring enzymes in the myocardium were examined and methods for the assay of the activity of these enzyme systems were developed. Aminoacyl-tRNA synthetase activity was measured from the rate of incorporation of ^{14}C -labelled amino acid into aminoacyl-tRNA. Transferase activity was measured from the rate of incorporation of amino[^{14}C]acyl-tRNA into protein in the presence of a standard preparation of hepatic ribosomes. Aminoacyl-tRNA synthetase activity is labile once the heart has been homogenized, whereas transferase activity is stable. The source of energy for synthetase activity is ATP; that for transferase is GTP. Transferase activity was inhibited by puromycin and stimulated by dithiothreitol, whereas synthetase activity was unaffected.

In recent years there has been an expanding interest in the control of protein synthesis in the heart during the development of cardiac hypertrophy (Meerson, 1969). Only limited attention has, however, been given to the physiological or pathological role of cytoplasmic factors in this process (Tomita, 1966; Moroz, 1967). The investigation of such a role depends on the availability of reliable methods of assay of the cytoplasmic enzymes that underlie the formation of proteins from amino acids. The present study has therefore been undertaken to establish methods of assay of aminoacyl-tRNA synthetase and aminoacyl-tRNA transferase activity in myocardial cytoplasm. The development of assay systems for these enzymes has added information about some of their properties.

Aminoacyl-tRNA synthetase activity catalyses the activation of amino acids and the aminoacylation of tRNA, and the aminoacyl-tRNA transferase enzymes transfer the aminoacyl group from aminoacyl-tRNA to nascent protein at the ribosomal site (Fessenden & Moldave, 1961). The present method for the assay of cytoplasmic aminoacyl-tRNA synthetase activity measures the rate of incorporation of ^{14}C -labelled amino acid into aminoacyl-tRNA in the presence of an excess of tRNA. That for aminoacyl-tRNA transferase activity measures the rate of incorporation of amino[^{14}C]acyl-tRNA into protein in the presence of a standard preparation of hepatic ribosomes. In addition the effect of cytoplasmic enzymes on the overall incorporation of ^{14}C -labelled amino acids into protein has been estimated. In the liver transferase activity has been shown to consist of two steps (Gasier & Moldave, 1965), although Earl & Morgan (1968) were unable to confirm this in cardiac muscle.

No attempt to separate these two stages has been made in the present study.

Experimental

Materials

L-[U- ^{14}C]Phenylalanine (477 mCi/mmol), L-[U- ^{14}C]leucine (344 mCi/mmol) and L-[U- ^{14}C]valine (260 mCi/mmol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. GTP, ATP, tRNA (brewer's yeast), poly(U), creatine phosphate and creatine phosphokinase were from Boehringer Corp. (London) Ltd., London W.5, U.K. Chemicals for liquid-scintillation counting were from Packard Instruments Ltd., Wembley, Middlesex, U.K. Dithiothreitol was from Calbiochem, Los Angeles, Calif., U.S.A., puromycin from Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A., Sephadex G-25 from Pharmacia (G.B.) Ltd., London W.5, U.K., and all other chemicals were obtained from BDH Chemicals Ltd., Poole, Dorset, U.K.

Sprague-Dawley male albino rats, weighing 150–180 g, were supplied by Scientific Products Farm, Canterbury, Kent, U.K., and New Zealand white rabbits, weighing 1.5–2 kg, were obtained from Laboratory Animals Suppliers, Whitecloud Farm, Tring, Herts., U.K.

Preparation of liver ribosomes

All the preparative procedures were carried out at 0–4°C. Rats were killed by a sharp blow on the head. The livers were dissected, washed with homogenizing

medium and minced with scissors. A 30% (w/v) homogenate was then prepared in an 0.2M-sucrose medium containing 0.02M-tris-HCl buffer, pH 8.2, 0.1M-KCl, 0.05M-NaCl, 6mM-magnesium acetate, 1mM-EDTA. Five strokes of a Teflon-glass homogenizer driven at 1500 rev./min were used for homogenization. The homogenate was centrifuged at 10000g for 10 min. The supernatant was carefully removed and kept in ice with 0.3% sodium deoxycholate and 0.25M-NH₄Cl (final concentrations) for 15 min. The sample was then layered over 1M-sucrose containing 0.02M-tris-HCl buffer, pH 7.6, 0.1M-KCl, 0.04M-NaCl, 6mM-magnesium acetate and 1mM-EDTA, and centrifuged at 105000g for 1.5 h. The upper phase was carefully aspirated, followed by the lower sucrose phase. The ribosomal pellet was stored at -15°C until required for use. For assay purposes each pellet was resuspended in 2 ml of resuspending medium (0.02M-tris-HCl buffer, pH 7.6, 0.1M-KCl, 0.04M-NaCl, 6mM-magnesium acetate, 1mM-EDTA, 6mM-2-mercaptoethanol, 0.25M-NH₄Cl) with gentle homogenization by hand with a Teflon pestle. The ribosomal pellets could be stored at -15°C for up to 1 month without any apparent loss in incorporation activity. The E_{260}/E_{280} ratio of the ribosomal suspension was 1.8.

The purity of ribosomal fractions was checked by electron microscopy, samples being fixed in 1% uranyl acetate by using a technique similar to that described by Burghouts *et al.* (1970). The electron micrographs showed ribosomes and polyribosomes with no apparent contamination by membrane or other cellular components.

Soluble fraction from myocardium

Rats and rabbits were killed by cervical fracture and the hearts quickly dissected and perfused with 5 ml of Krebs-Henseleit solution (Krebs & Henseleit, 1932) at 4°C. Perfusion was performed by cannulating the aorta with a tube (1 mm diam.) attached to a 5 ml syringe from which the fluid was gently expelled. The hearts were minced with scissors and washed with homogenizing medium. A 25% (w/v) homogenate was prepared by using a Teflon-glass homogenizer for rats or an Ultra-Turrax homogenizer for rabbits.

The homogenates were centrifuged at 105000g for 2 h. The supernatant was carefully removed and passed through a Sephadex G-25 column, the bed volume of which was at least 10 times greater than the volume of supernatant (Earl & Korner, 1965). The Sephadex G-25 was prepared by boiling for 1 h in a water bath, cooling and washing with 0.02M-tris-HCl buffer, pH 7.6, containing 0.25M-NH₄Cl and 6mM-MgCl₂. The Sephadex G-25 was equilibrated with 50 ml of the resuspending medium used for ribosomes before the soluble fraction was applied. To check that contaminating amino acids were

efficiently removed by the Sephadex G-25 column, ¹⁴C-labelled protein hydrolysate with a total activity of 130000 c.p.m. was added to 2 ml of myocardial cytoplasmic fraction and passed through the column. There was no detectable radioactivity in the protein fraction of the effluent.

Soluble-fraction protein was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard.

Preparation of [¹⁴C]phenylalanyl-tRNA

This was prepared from rat liver supernatant fraction by the method of Moldave (1967). The sample was kept at -15°C until required for use. The radioactivity of the sample was approx. 180000 c.p.m./mg of RNA and could all be precipitated with ice-cold 0.5M-HClO₄.

Assay of incorporation of ¹⁴C-labelled amino acids into protein in vitro

All solutions and samples were kept at 0-4°C, except for incubation of the assay mixture. The reaction mixture contained, in a total of 1 ml, 0.02M-tris-HCl buffer, pH 7.6, 0.04M-NaCl, 0.01M-KCl, 1mM-MgCl₂, 5mM-ATP (disodium salt containing equimolar MgCl₂; pH adjusted to 7.6), 0.2mM-GTP, 10mM-creatine phosphate, 10 μg of creatine phosphokinase, 20mM-dithiothreitol, 0.5 μCi of ¹⁴C-labelled amino acid, 0.05 μmol of 19 unlabelled amino acids (excluding the amino acid added as the ¹⁴C source) and 0.3-0.5 mg of ribosomal-suspension protein. The reaction was started by the addition of soluble fraction (0.6-1 mg of soluble-fraction protein). The reaction mixture was incubated at 37°C and stopped by the addition of 5 ml of 0.5M-HClO₄ containing 1 mg of unlabelled amino acid/l. The mixture was boiled in a boiling-water bath for 15 min and then centrifuged. The precipitate was washed three times with 0.5M-HClO₄ containing labelled amino acid and then filtered through a glass-fibre Whatman GFA filter and washed with HClO₄, ethanol and ether. When dry the filter was placed in 5 ml of dioxan scintillant [containing 5 g of 2,5-diphenyloxazole and 0.3 g of 1,4-bis-(5-phenyloxazol-2-yl)benzene/l] and counted for radioactivity in a Packard liquid-scintillation counter for 10 min or 10000 counts, whichever was completed first. A wash with chloroform-methanol to remove lipid was included in some early experiments but was found to be unnecessary.

Enzyme assays

Aminoacyl synthetase. The reaction mixture contained, in a total volume of 1 ml, 0.02M-tris-HCl

buffer, pH7.6, 0.04M-NaCl, 0.01M-KCl, 11mM-MgCl₂, 5mM-ATP (as described above), 10mM-creatine phosphate, 10μg of creatine phosphokinase, 0.5μCi of ¹⁴C-labelled amino acid, 0.05μmol of 19 unlabelled amino acids (except the amino acid added as the labelled compound) and 200μg of tRNA. The reaction was started with the addition of 0.2–0.3mg of soluble-fraction protein. The reaction mixture was incubated at 37°C and stopped by the addition of 5ml of ice-cold 0.5M-HClO₄ containing unlabelled amino acid. The tubes were kept in ice for 10min and then centrifuged at 4°C. The precipitate was washed twice with ice-cold HClO₄, filtered and counted for radioactivity as described above.

Assay of transferase activity. For the assay of transferase enzyme activity a 1ml assay mixture contained: 0.02M-tris-HCl buffer, pH7.6, 0.04M-NaCl, 0.01M-KCl, 11mM-MgCl₂, 0.2mM-GTP, approx. 16000c.p.m. of [¹⁴C]phenylalanyl-tRNA and 0.3–0.5mg of ribosomal-suspension protein. The reaction was started by the addition of 0.2–0.3mg of soluble-fraction protein. The incubation temperature was 37°C. The reaction was stopped, and the products were processed and counted for radioactivity as described under the assay measuring incorporation of ¹⁴C-labelled amino acid into protein.

Results

Incorporation of amino acids into protein

Table 1 shows the results of a series of experiments demonstrating the ability of rat liver ribosomes and

Table 1. *Effects of varying the composition of the incorporating system in vitro on incorporation of [¹⁴C]phenylalanine into protein by rat and rabbit myocardial soluble fraction*

Assay details are given in the Experimental section except for the addition and omissions noted below. The incubation time was 15 min. Results, expressed as c.p.m. of [¹⁴C]phenylalanine incorporated/mg of soluble-fraction protein, are the means of duplicate experiments. Rat liver ribosomes were used throughout the experiments.

	Incorporation	
	Rat	Rabbit
Standard assay system	16600	13960
ATP-regenerating system omitted	8120	9795
ATP omitted	900	1580
GTP omitted	278	1630
Poly(U) added	24500	36248
Puromycin added	1470	1800
20mM-Dithiothreitol added	25000	15360
Amino acid mixture omitted	11670	9493

rat heart soluble factors to incorporate ¹⁴C-labelled amino acid into protein. Without the addition of soluble factors there was no incorporation. An assay without ATP or GTP had a considerably decreased incorporating ability. Poly(U) stimulated the incorporation of [¹⁴C]phenylalanine approximately two-fold. Addition of 20mM-dithiothreitol increased the ¹⁴C-labelled amino acid uptake by 20%. Puromycin decreased the incorporation to 10% of the control value. Omission of the ATP-regenerating system (creatine phosphate and creatine phosphokinase) decreased incorporation considerably. Omission of amino acids caused a decrease in incorporation. The optimum concentration of amino acids under our assay conditions was 0.02–0.03μmol (Fig. 1). Addition of tRNA up to 200μg had no effect on the incorporation of amino acid into protein.

Although ribosomal pellets could be stored at –15°C for up to 1 month without loss of activity, the overnight storage of soluble fraction at –15°C caused a 60% loss of incorporating ability. Freeze-drying of rat heart soluble fraction caused a considerable fall in activity, which decreased even further on storage (Fig. 2). Freezing and thawing of the myocardial soluble fraction had an adverse effect on the synthetic activity. The activity of a fresh sample assayed in triplicate was 6663c.p.m./mg of soluble-fraction protein. It was 6140 when frozen and thawed once, and 5283 and 4013 after further successive freezing and thawing.

By contrast, soluble fraction obtained from hearts kept for up to 3h at either 0°C or at room temperature (21°C) did not show any loss in incorporating activity in the system *in vitro*.

Fig. 1. *Effect of amino acid concentration on the uptake of phenylalanine into protein by a rat heart soluble fraction*

Incubation conditions are given in the text except that increasing concentrations of amino acids, from 0.02 to 0.3μmol, were added. Each point is the mean of triplicate experiments.

Fig. 2. Incorporation of amino acid into protein by rat heart soluble fraction after freeze-drying and subsequent storage at -15°C

Preparation of the soluble fraction and the assay procedure are described in the Experimental section. Each point is the mean value of triplicate experiments.

Rat heart soluble fraction gave a linear time-activity relationship up to 20 min, after which the incorporation ceased to follow linear kinetics. Linearity could be restored after a 30 min incubation by the addition of more ribosomes or poly(U) but not with more soluble fraction (Fig. 3). Similar results were obtained with rabbit heart soluble fraction.

Synthetase activity

Fig. 4 shows that with the synthetase assay system there was a linear incorporation of labelled amino acid into aminoacyl-tRNA up to 10 min with 0.35 mg of soluble protein. With lower concentrations of tRNA, linearity was not maintained for so long. When twice as much soluble-fraction protein was added the incorporation was doubled up to 5 min, after which the reaction became non-linear as the availability of tRNA became limiting. When tRNA was omitted from the reaction mixture the time-activity curve shown in Fig. 5 was obtained. The ^{14}C -labelled amino acid uptake was considerably decreased without the added tRNA and the time-activity curve was not linear. The synthetase reaction was dependent on ATP and amino acid uptake was increased by the addition of an ATP-regenerating system (Fig. 5). CTP or GTP could not replace ATP. Dithiothreitol up to 20 mM had no effect on synthetase activity. Puromycin (0.2 mM) had very little effect on synthetase activity, decreasing uptake from 21.7 to 18.8 μmol of phenylalanine incorporated/mg of protein (means of three experiments).

Fig. 3. Incorporation of [^{14}C]phenylalanine into protein by rat myocardial soluble fraction and liver ribosomes

Preparation and assay details are given in the Experimental section. ●, Complete assay system without any further additions at 30 min; ○, 0.35 mg of ribosomal fraction added at 30 min; ▲, 200 μg of poly(U) added at 30 min. Each point is the mean of duplicate experiments. Arrow indicates time of additions.

Table 2 shows the results of adding various concentrations of soluble fraction. Within the range of protein added there was a linear increase in ^{14}C -labelled amino acid uptake with increasing protein concentration. Storage of the soluble fraction overnight at -15°C resulted in a 90% loss of ^{14}C -labelled amino acid uptake. The rabbit heart soluble fractions gave similar results except that the uptake was 50% less than that shown by the rat.

There appears to be a considerable variation in the activity of different amino acid-specific synthetases and in the concentrations of specific tRNA in the myocardial cell sap. Fig. 6 shows that, in the absence of added tRNA, the rate of formation of leucyl-tRNA was greater than either phenylalanyl-tRNA or valyl tRNA. When 200 μg of yeast tRNA was added, the rate of formation of all three forms of aminoacyl-tRNA was increased, but that of phenylalanyl-tRNA was considerably greater than that of leucyl-tRNA. The observations suggest that there is a greater quantity of phenylalanine-specific synthetase present compared with leucine-specific synthetase, although there is normally a greater quantity of leucine-specific tRNA than phenylalanine-specific tRNA in rabbit heart soluble preparations. Valine uptake remained low in both the presence and the absence of added

Fig. 4. Time-activity relationship of rat myocardial aminoacyl synthetase activity with added tRNA

The full assay conditions are given in the Experimental section. With 0.35 mg of soluble-fraction protein various concentrations of tRNA were added: ●, 50 μ g; ○, 100 μ g; ▲, 150 μ g; △, 200 μ g; □, 200 μ g of tRNA and 0.7 mg of soluble-fraction protein. Each point is the mean of duplicate experiments.

Fig. 5. Time-activity relationship of rat myocardial aminoacyl synthetase activity without added tRNA

The assay mixture contained, in 1 ml, 0.02 M-tris-HCl buffer, pH 7.6, 0.04 M-NaCl, 0.01 M-KCl, 11 mM-MgCl₂, 5 mM-ATP, 10 mM-creatine phosphate, 10 μ g of creatine phosphokinase, 0.5 μ Ci of [¹⁴C]phenylalanine, 0.05 μ mol of 19 unlabelled amino acids (●). ○, ATP-regenerating system omitted; △, without added ATP; ▲, 7.5 mM-ATP added. The results are means of duplicate experiments.

tRNA (rich in valine-specific tRNA), suggesting a low concentration of valine-specific synthetase activity.

Transferase activity

No attempt was made in the present study to separate the transferase I and II components reported by Gasier & Moldave (1965). The time-course of [¹⁴C]phenylalanine transfer from [¹⁴C]phenylalanyl-tRNA into protein shows that the reaction was linear up to 15 min (Fig. 7). The transfer was almost completely inhibited by omitting GTP. ATP could not be substituted for GTP. Poly(U) stimulated transferase activity to a value approx. 3 times that of the control activity. Some residual transferase activity remains tightly bound to ribosomal preparations (Skogerson & Moldave, 1968). In our ribosomal preparations the residual transferase activity was sometimes as high as 30% of the total transfer activity. Puromycin inhibited transfer activity by 75%. Addition of 20 mM-dithiothreitol to the assay system doubled the rate of transferase activity.

Transferase activity was not affected by storage of the soluble fraction overnight at -15°C. Table 3 shows the effects of increasing concentrations of soluble enzyme. The residual ribosomal transferase

activity has been subtracted from the results. It seems that the relation between activity and concentration of soluble-fraction protein is reasonably linear up to a concentration of 0.1 mg of soluble fraction per assay system. Above this concentration the increased activity became progressively less. The limitation in activity was not due to a lack of GTP or to a lack of amino[¹⁴C]acyl-tRNA, since the addition of further quantities of these substances had no effect on activity.

Discussion

Synthetase activity in the myocardial cytoplasm is evidently labile once the heart has been homogenized, but activity remains unaffected by storage of the whole heart for up to 3 h. The source of energy for synthetase activity is ATP. The role of individual aminoacyl-tRNA synthetases remains unexplored, but it is clear that there is a considerable variation in the activity of different synthetase in the myocardium.

In contrast with synthetase activity, transferase activity in the fractionated cytoplasm appears to be stable. The source of energy for transferase activity has been found to be GTP in the present study. This

Table 2. *Effects of adding various concentrations of heart soluble fraction on aminoacyl synthetase activity*

Details of the assay of aminoacyl synthetase activity are given in the Experimental section. The incubation time was 2 min or 5 min. Results are expressed as pmol of phenylalanine incorporated/mg of soluble-fraction protein and are the means ($\pm$ S.D.) of three experiments.

Animal	Soluble-fraction protein added (mg)	Incubation time ...	Incorporation	
			2 min	5 min
Rat	0.4		5.26 ± 0.94	14.38 ± 2.44
	0.6		5.20 ± 0.53	12.94 ± 1.37
	0.8		5.70 ± 0.32	11.24 ± 1.28
Rabbit	0.5		3.32 ± 0.32	8.67 ± 1.40
	0.7		3.84 ± 0.10	9.68 ± 0.70
	0.9		3.88 ± 0.11	8.63 ± 0.81

Fig. 6. *Rabbit heart aminoacyl synthetase activity*

The assay conditions are described in the Experimental section. Enzyme activity was measured with [14 C]-phenylalanine (■), [14 C]leucine (●) and [14 C]valine (○) in the absence (a) and presence (b) of additional RNA (200 μ g).

agrees with the observations of Fessenden & Moldave (1963) in the liver but not with those of Earl & Morgan (1968), who were unable to find a requirement for GTP in a myocardial system. The amount of residual transferase bound to the ribosomal preparation was very variable, so that it is important to measure this separately for each assay. The reason for the non-linearity of the relation between activity and quantity of cytoplasm when larger amounts of cytoplasm are added is not clear. It does not appear to be due to any lack of GTP or aminoacyl-tRNA or ribosomes. Neither would it seem likely to be due to a

deficiency in any other factor present in the cytoplasmic or ribosomal fractions.

Although full aminoacyl-tRNA synthetase activity required the addition of excess of tRNA, the complete protein-synthesizing system was fully active in the presence of its own intrinsic tRNA. This is presumably due to the cyclical usage of tRNA in the cytoplasm (Nathans & Lipmann, 1961; Fessenden & Moldave, 1961; Noll *et al.*, 1963).

The diminution of activity of the total protein-synthesizing system after 20 min incubation would seem to be due to the destruction of mRNA, since it

Fig. 7. Amino acid-transferring enzyme activity of rat heart soluble fraction

The assay mixture is given in the Experimental section. ●, Complete assay system; ○, system minus soluble fraction. The results are the means of duplicate experiments.

could be restored by the addition of poly(U) or more ribosomes. Presuming that this was due to ribonuclease activity, we assayed the ribonuclease activity of the cytoplasmic fraction (Shortman, 1961). The maximal ribonuclease activity found by this method was, however, only 0.005 unit/mg of cytoplasmic protein.

Puromycin had little effect on cardiac aminoacyl-tRNA synthetase activity, but inhibited 75% of the transferase activity. Conversely dithiothreitol (20mM) was found greatly to stimulate transferase activity but to have no effect on synthetase activity. The stimulating effect of this substance on transferase activity is similar to that described by Sutter & Moldave (1966) in the liver. Earl & Morgan (1968), however, had been unable to find such an effect in the heart.

The calculation of the number of pmol of phenylalanine converted into phenylalanyl-tRNA/min per mg of cytoplasmic protein gives a measure of specific phenylalanyl-tRNA synthetase activity. It presupposes that the concentration of phenylalanine-specific tRNA is not limiting, and the linear incorporation of [14 C]phenylalanine supports this supposition. On the other hand it does not give an indication of overall synthetase activity, which is the composite of twenty specific aminoacyl-tRNA synthetases. From the evidence presented above it would seem that there are considerable differences in the activity of the individual synthetases, neither can one be completely sure that the supply of specific tRNA is not a limiting factor in each case. There is therefore no entirely satisfactory way of expressing total aminoacyl-tRNA synthetase activity from the

Table 3. Effect of adding increasing concentrations of soluble enzyme and ribosomes on myocardial transferase enzyme activity

The assay system is described in the Experimental section and the incubation time was 15 min. Results are expressed as c.p.m. of [14 C]phenylalanine transferred from [14 C]phenylalanyl-tRNA to protein and are the means of triplicate experiments.

Soluble enzyme added (mg)	Concn. of ribosomal protein ...	Transferase activity	
		0.3mg	0.6mg
0.06		1563	1511
0.12		2950	2938
0.15		3144	3172

measurement of the rate of incorporation of one amino acid.

The calculation of total aminoacyl-tRNA transferase activity from the rate of incorporation of phenylalanyl-tRNA into polypeptide also presents difficulties, since the rate of incorporation of the individual labelled amino acid is dependent not only on transferase activity but also on the amino acid composition of the polypeptide that is being formed. This in turn is dictated by the particular message that is carried on the mRNA associated with the hepatic ribosomal fraction. Thus, as has been shown in the present study, if the nature of the mRNA is changed by adding poly(U) the rate of incorporation of phenylalanine into polypeptide can be increased while the availability of cytoplasmic transferase enzymes is held constant and presumably the total rate of protein synthesis remains unchanged. The calculation of total transferase activity from the specific radioactivity of mixed aminoacyl-tRNA labelled with [14 C]phenylalanine would have to assume that the proportion of phenylalanine in the polypeptides that are being formed is the same as the proportion of phenylalanyl-tRNA in the mixture of tRNA. Any calculation of the total rate of flow of amino acid into polypeptide under the influence of both synthetase and transferase activity would also depend on the assumption (equally unlikely to be strictly true) that the proportion of phenylalanine in the polypeptide is the same as the proportion of phenylalanine in the mixture of free amino acids.

We have therefore preferred to express transferase and synthetase-plus-transferase activities simply in terms of c.p.m. incorporated into polypeptide. The activity expressed in this way is specific for a particular ribosomal preparation. It is therefore not absolute. On the other hand, it will allow a comparison between the cytoplasmic enzymes of different myocardia. The stability of the hepatic ribosoma

preparation is of practical importance in this respect.

Within the limitations imposed by the above theoretical considerations the present methods give a satisfactory estimation of synthetase activity and transferase activity and of the total protein-synthetic system linear with time and with quantity of cytoplasm. The need for such quantitative methods is imperative in the study of those alterations in protein synthesis in the myocardium that accompany chronic changes in mechanical work, the supply of oxygen or the hormonal environment. It was with these ends in mind that the present techniques of assay were established.

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