J. Biosci., Vol. 2, Number 3, September 1980, pp. 243-251. © Printed in India.

Age-related qualitative and quantitative changes in tRNA population of rat skeletal muscle

MANJULA and R. M. SUNDARI

Biochemistry Laboratory, Department of Zoology, Banaras Hindu University, Varanasi 221 005,

MS received 21 March 1980; revised 12 May 1980

Abstract. Total tRNA was purified from skeletal muscle of young, adult and old female albino rats. Age-dependent variation of total tRNA was the same with respect to tRNA content and biological activity as measured by amino acid acceptor capacity. The tRNA content was more in young rats and showed a gradual decrease in the adult and old rats. The relative abundancy of eleven aminoacyl-tRNAs were checked at each age and during aging. Arginyl, glutamyl and tyrosyl-tRNAs do not show any quantitative or qualitative change with age.

Keywords. Rat; skeletal muscle; tRNA; aging; aminoacylation; aminoacyl-tRNA fractionation.

Introduction

Age-related variation in tRNA population has already been reported in lower organisms (Reitz and Sanadi, 1972; Hoffman, 1972; Hoffman and McCoy, 1974), plants (Shugart, 1972; Bick *et al*., 1970; Mettler and Romani, 1976; Palatnik and Katz, 1977) and animals (Portugal, 1972 a, b; Daikee, 1976; Mays *et al*., 1978). Similar investigations made by other workers revealed no change in isoacceptor species of tRNA with age (Frazer and Yang, 1972; Klee *et al*., 1978). In general, changes are observed only in a few specific tRNAs in any system. A regulatory mechanism, was proposed for these changes in the quantity and structure of specific tRNAs in the translation process, which in turn controls cellular differentiation, development and aging.

In the present work, a study of the age-related changes in tRNA population in skeletal muscle of female albino rats was undertaken. Female rats of three age groups representing pre-reproductive (6-8 weeks), reproductive (38-40 weeks) and post-reproductive (78-80 weeks) phases of the life span were used. These groups are referred to as young, adult and old respectively. Transfer RNA was isolated from skeletal muscle to compare the total tRNA population at all the three age-groups. Following the isolation of tRNAs and aminoacyl-tRNA synthetases, transfer RNAs

Abbreviations used: EGTA: ethyleneglycoltetraacetic acid.

were aminoacylated with 11 amino acids to assay for quantitative changes in the acceptor activity for each of these amino acids during growth and aging. Finally qualitative changes in the isoacceptor species of arginyl, glutamyl and tyrosyl-tRNAs were analyzed using DEAE-sephadex column chromatography.

Materials and methods

Female albino rats of Wistar strain were used for the studies. Skeletal muscle from legs and arms from 8-10 rats of each age group were pooled and used as starting material to isolate total tRNAs.

Isolation of total tRNA

Transfer RNA was extracted in identical fashion from skeletal muscle of young, adult and old rats according to the method of Dure (1973) with some modification. All the steps were carried out at 4°C unless otherwise specified. The tissue was treated twice with 0.005 M phosphate buffer, pH 6.8, 0.1M KCl, 0.005M $MgCl_2$, 0.005M EGTA before homogenization according to the method of Sherton and Wool (1974). Calcium ions are removed from the muscle by the chelating agent EGTA causing its relaxation and thus easier and efficient homogenization was achieved. Tissue was homogenized in 0.01 M Tris-HCl buffer, pH 7.5, 0.01M KCl, 0.01M $MgCl_2$, 0.001M EDTA, 1% sodium deoxycholate and 0.001M 2-mercapto-ethanol. Homogenate was centrifuged at 14,000 *g* for 15 min and the pellet was re-extracted. The supernatant was treated thrice with equal volume of phenol for 30 min. The aqueous layer was made 3% with potassium acetate, pH 5.0 and precipitated with 2.5 volume ofice-cold ethanol. The precipitate was recovered by centrifugation at 14,000 *g* for 15 min and dissolved in 0.01M Tris-HCl buffer, pH 7.5, 0.01M $MgCl_2$, 0.25M NaCL Sample was loaded on to DEAE-cellulose column (15 cm × 1.2 cm) pre-equlibrated with the same buffer.Transfer RNA was eluted from the column using 1.0M NaCl in the same buffer. Fractions containing tRNA were pooled and tRNA was precipitated with ice-cold ethanol. Total tRNA was loaded on to a Sephadex G-100 column (75 cm × 1 cm) previously equilibrated with 0.01M Tris-HCl buffer, pH 7.5, 0.4M NaCl, 0.01M $MgCl_2$ and eluted with the same buffer. This step was carried out at 25°C. The purified tRNA after gel filtration eluted in the range of standard *Escherichia coli* tRNA. The fractions were pooled and precipitated with ice cold ethanol to recover tRNA. Transfer RNA was always deacylated before aminoacylation reaction.

DNA and RNA concentration of the. homogenate were estimated according to the method of Schneider (1957).

Preparation of aminoacyl-RNA synthetases

Aminoacyl-tRNA synthetases from young, adult and old rate tissue were purified according to the method of Yang and Novell (1971), with some modifications. Fresh tissue was homogenized in 0.01M Tris-HCl buffer, pH 7.6, 0.005M magnesium acetate, 0.01M KCl, 0.005M EDTA, 0.01M 2-mercaptoethanol and centrifuged at 14,000 *g* for 15 min at 4°C. The clear supernatant was centrifuged at 150,000 *g* for 3 h in an ultracentrifuge (Vac 601). The supernatant was applied onto a DEAE-cellulose column (15 cm × 1 cm) which had been previously equilibrated at 4°C with

phosphate buffer, pH 7.6, 0.001M $MgCl_2$, 0.002M dithiotreitol and 0.005M KCl.The aminoacyl- tRNA synthetases were eluted with the same buffer containing 0.3M KCl. Fractions having synthetase activity were dialyzed against 0.05M Tris-HCl buffer, pH 7.5, 0.005M EDTA and 0.002M dithiotreitol. Aliquots (0.5 ml) were stored frozen at –20°C until use. The column step was necessary to remove endogenous tRNAs and free amino acids from the enzyme preparation. The enzyme protein was estimated according to the method of Lowry *et al*. (1951) using bovine serum albumin as the standard.

Aminoacylation

Amino acid acceptor activity of tRNA was determined by measuring the incorporation of radioactive amino acids into trichloroacetic acid insoluble material using the paper disc assay method of Mans and Novelli (1961). The reaction mixture contained 100 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 10 mM KCl, 10 mM NH_4Cl, 4 mM 2-mercaptoethanol, 2 mM ATP, 1 mM CTP, 2.0 A_{260} units of tRNA and 0.3 mg of enzyme protein (from homologous source) in a total volume of 0.3 ml [^{14}C]-algal protein hydrolysate (0.17 *u*Ci) from Bhabha Atomic Research Centre, Trombay, Bombay was used as a source of total amino acids to assay total amino acid acceptor capacity of tRNAs. The reaction was carried out for 25 min at 30° C and then terminated by trichloroacetic acid addition. The precipitate was collected and counted in a toluene-based scintillation fluid containing 4 g of 2,5-diphenyl-oxazole (PPO) and 0.1 g of 1,4-bis [2-(5-phenyloxazoyl)] benzene (POPOP) per litre of toluene.

Preparation of aminoacyl-tRNA

For chromatographic studies, the total volume of the reaction mixture was scaled up. Following aminoacylation of tRNAs for 25 min, the reaction mixture was diluted with 2.6 volumes of 0.005 M sodium acetate buffer, pH 5.0, 0.1 M NaCl and cooled in an ice-bath. The sample was adsorbed onto asmall DEAE-cellulose column (1ml bed volume) pre-equilibrated with the same buffer. Aminoacyl-tRNA was eluted with 2.0M NaCl in the same buffer and recovered by precipitation with ice-cold ethanol. The precipitate was dissolved in 0.02M sodium acetate buffer, pH 4.5, 0.50 M NaCl and 7 M urea and stored at –20° C until use. Using this procedure, [^{14}C] aminoacyl-tRNAs were prepared from muscle of adult and old rats and [^{3}H] aminoacyl-tRNAs from that of young rats. Aminoacyl-tRNAs from young and adult or from young and old rats were mixed and co-chromatographed on a DEAE Sephadex column.

Cochromatography on DEAE-Sephadex column

The mixed tRNA sample was applied onto a DEAE-Sephadex column (60 cm×1 cm) and the column was developed at room temperature using a linear 140 ml sodium chloride gradient ranging from 0.50 M to 0.66 M in 0.02 M acetate buffer, pH 4.5, 7 M urea. Fractions of 0.8 ml were collected at a flow rate of 4 ml/h. Aliquot of the fractions (0.5 ml) were applied on Whatman number 1 filter paper strips (2.5 cm×5 cm). The strips were dried under infrared lamp and washed twice with 75% ethanol containing 0.1 M NaCl to remove urea, dried and counted in a toluene based scintillation fluid as mentioned earlier.

Results

Table 1 shows age-related variation in tRNA content (based on their absorbancy at 260 nm) of skeletal muscle. When expressed on a weight basis, 20 absorbance units at 260 nm were assumed to be equivalent to one mg of tRNA. Transfer RNA content was maximum inyoung tissue. It showed a gradual decrease with age, the lowest content being in old age. This decrease from the young to adult and from adult to old is quite significant when expressed as μg tRNA per mg of DNA which is the best parameter to express nucleic acid content of any tissue. The same pattern of variation was observed when the data was expressed per g weight of tissue, per mg RNA or per g protein.

Table1. Age related variation in tRNA content of female rat muscle.

Unit of measurement	Age of rats		
	7 weeks	40 weeks	80 weeks
μ g tRNA/g wet weight	44.60± 0.60^a	40.00±1.00^d	39.60± 0.50
μ g tRNA/mg DNA	67.40± 0.20^b	62.40±0.60^c	58.80± 1.70
μ g tRNA/g protein	1246.00±15.00^b	990.00±9.80^d	970.00±10.00
μ g tRNA/mg RNA	61.10± 1.40^d	58.80±1.50^b	45.80± 0.30

'*p*' value is calculted between young and adult and adult and old.
[a] $P< 0.01$; [b] $P< 0.001$; [c] $P< 0.05$; [d] not significant.

The variation in the total amino acid acceptor capacity of tRNAs from young, adult and old tissues was measured using [^{14}C]-algal protein hydrolysate as the source of labelled amino acids and aminoacyl-tRNA synthetases from homologous sources. Total amino acid acceptor activity of tRNAs showed a similar pattern of variation with age as found in the case of total tRNA content (figure 1). This showed that total tRNA isolated from each tissue retained biological activity throughout the experimentation. The acceptor activity of tRNA was highest in the young and it decreased with age reaching the lowest levels in the old. When expressed per mg DNA, a similar variation pattern was observed.

Figure 2 shows amino acid acceptor activities of tRNAs of skeletal muscle from the three different ages when tested with eleven amino acids.

In general, there was a quantitative variation in the different aminoacyl-tRNAs at each age. Tyrosyl, arginyl; lysyl, alanyl, aspartyl-tRNA were in higher concentrations, whereas leucyl and prolyl-tRNAs were present in quite low amounts. The relative abundancy of these aminoacyl-tRNAs in muscle may be due to the preferential synthesis of muscle proteins. A number of studies showed that the tRNA complement of some tissues, specialized for the production of one or a few proteins, reflected the amino acid composition of that protein (Garel *et al*., 1970, 1971: Chavancy *et al*., 1971; Elska *et al*., 1971; Smith and McNamara, 1972; Lanks and Weinstein, 1970; Maepaa and Ahonen, 1972). The proteins of the muscle, i.e.,

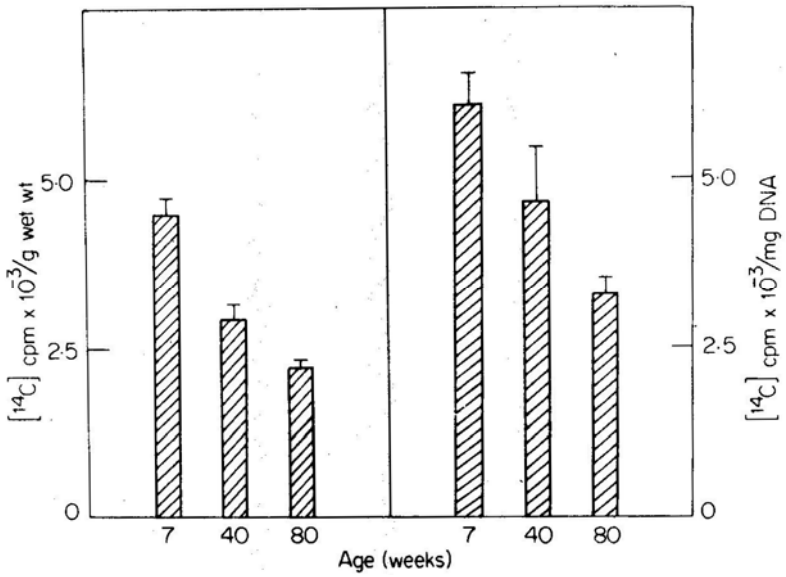

Figure 1. Transfer RNA from young, adult and old rat muscle was aminoacylated using aminoacyl-tRNA synthetases from young, adult and old rat muscle respectively. [^{14}C]-Algal protein hydrolysate was used as a source of amino acids.

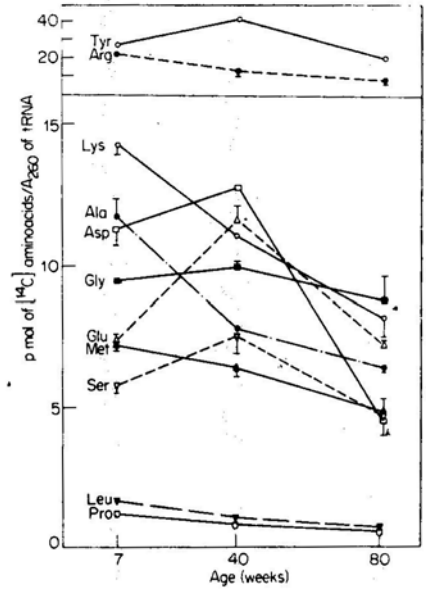

Figure 2. Transfer RNAs from skeletal muscle of young, adult and.old rats were assayed using homologous aminoacyl-tRNA synthetases. Specific activities of [^{14}C]-amino acids used were as follows:
Tyr 40 mCi/mmol; Arg 150 mCi/mmol; Gly 50 mCi/mmol; Ser 75 mCi/mmol;
Ala 75 mCi/mmol; Asp 100 mCi/mmol; Gly 125 mCi/mmol;
Pro 125 mCi/mmol; Leu 150 mCi/mmol; Lys 150 mCi/mmol; Met 54 mCi/mmol.

myosin, actin, troponin and tropomyosin of rabbit were reported to be rich in glutamic acid, aspartic acid, leucine and lysine (Laki, 1971; Bodwell, 1971). Contrary to rabbit muscle proteins, the relative proportion of leucine in rat muscle proteins must be very low since leucyl-tRNA concentration was found to be very low in rat muscle.

Fractionation of arginyl, glutamyl and tyrosyl-tRNAs are shown in figures 3,4 and 5. Though arginyl and glutamyl-tRNAs fractioned into different isoacceptor species neither qualitative nor quantitative changes were found in the distribution of iso-acceptor species with age. Tyrosyl-tRNA did not fractionate under the experimental conditions used. The elution profile showed two shoulders which may represent two more isoacceptors. No major changes were found in the tyrosyl-tRNA elution profile as a function of age.

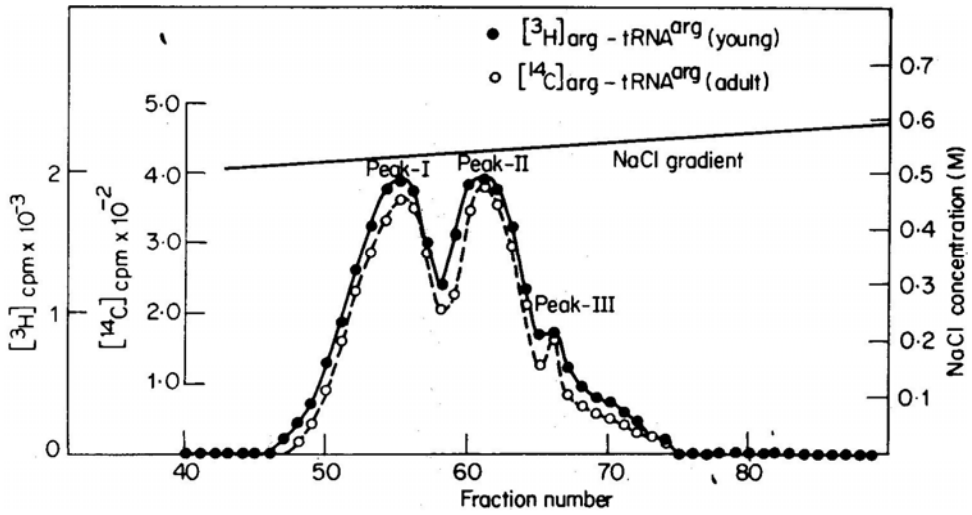

Figure 3. [^{14}C]-Arginine (354 mCi/mmol) and [^{3}H]-arginine (8.5 Ci/mmol) were used. In figure 3, [^{3}H]-arginyl-tRNA from young rat was mixed with [^{14}C]-arginyl-tRNA from adult and co-chromatographed. The column was developed as mentioned in material and methods. A similar fractionation pattern was observed when [^{3}H]-arginyl-tRNA from young rat was mixed and co-chromatographed with [^{14}C]-arginyl-tRNA from old. Percentage recovery was 85-95%. Peaks, I, II and III contain about 50%, 40% and 10% of total arg-tRNA ^{arg} respectively in all the three ages.

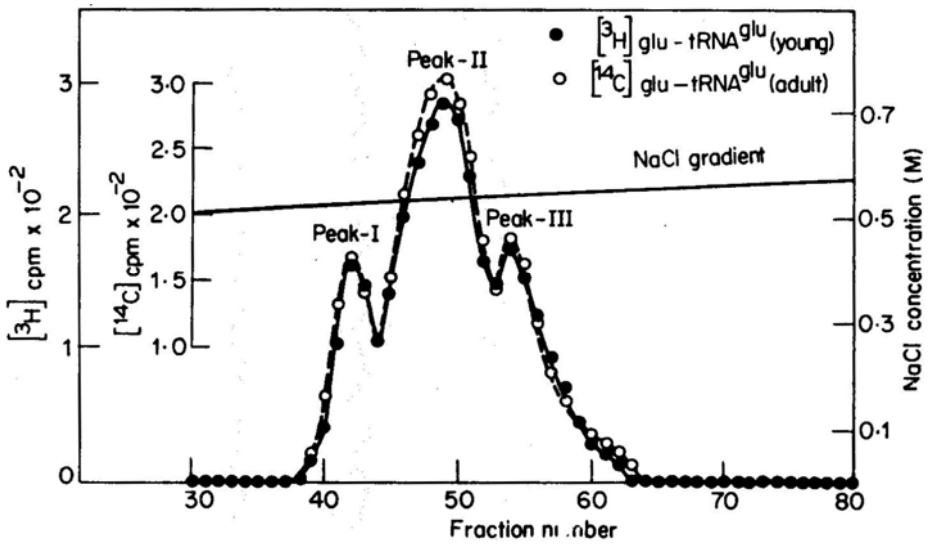

Figure 4. [^{3}H]-Glutamic acid (1.77 Ci/mmol) and [^{14}C]-glutamic acid (284 mCi/mmol) were used [^{3}H]-Glutamyl tRNA from young was mixed with [^{14}C]-glutamyl-tRNA from adult in figure 4 and co-chromatographed. The column was developed as mentioned in materials and methods. Glutamyl-tRNA from old showed a similar pattern of fractionation of isoacceptor species.

The precentage recovery was 78-96%. Peaks I, II and III contain about 20%, 60% and 20% of glu-tRNA ^{glu} respectively in all the three ages.

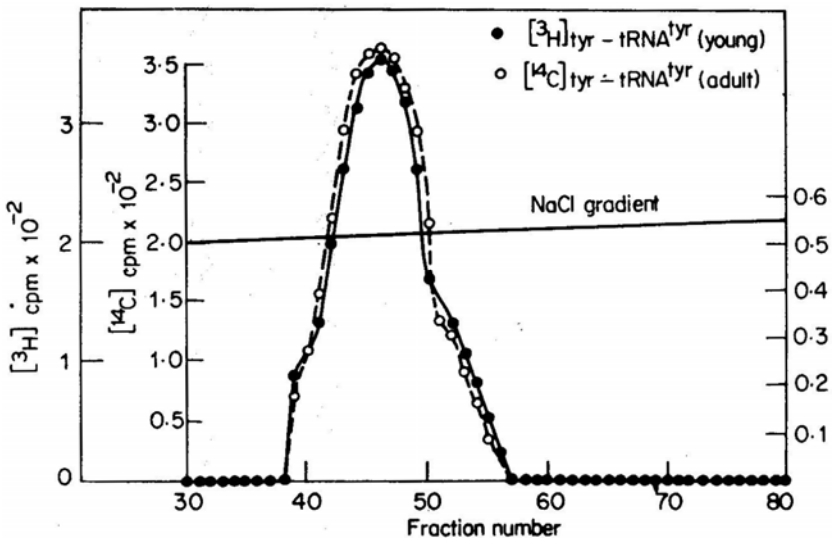

Figure 5. [^{3}H]-Tyrosine (2 Ci/mmol) and [^{14}C]-tyrosine (518 mCi/mmol) were used. [^{3}H]-tyrosyl-tRNA from young and [^{14}C]-tyrosyl-tRNA from adult was mixed for chromatography. The column was developed as mentioned in materials and methods. Percentage recovery was 90-98%. Tyrosyl-tRNA from old rat tissue showed a similar fractionation pattern.

Discussion

Total tRNA content was maximum in the skeletal muscle of the young rats. Skeletal muscle is a post-mitotic tissue and its development continues upto about 50 days. The concentration of transfer RNA was maximum and it was also maximally active during developmental phases of the tissue, after which it showed a gradual decline with age.

The isoacceptor species of three aminoacyl-tRNAs did not show any qualitative or quantitative changes. Skeletal muscle, being a post-mitotic tissue, may not need any drastic changes in the isoacceptor species of tRNAs since there may not be too many different types of messengers appearing with age. All the isoacceptor species of these aminoacyl-tRNAs may be used for protein synthesis in young, adult and old rat muscle. The other possibility is that arginyl, glutamyl and tyrosyl-tRNAs may not be involved in regulation brought about by transfer RNA in general. In other words these aminoacyl-tRNAs may not be the modulating tRNA species. There may be other aminoacyl-tRNAs present in skeletal muscle of rats which act as modulating species of tRNA thereby regulating protein synthesis and consequently development and ageing. Whether any qualitative or quantitative changes are present in other aminoacyl-tRNAs of skeletal muscle remains to be studied.

Acknowledgement

The investigation was supported by University Grants Commission, New Delhi.

References

Bick, M. D., Liebke, H., Cherry, J, H. and Strehler, B. L. (1970) *Biochim. Biophys. Acta*, **204,** 175.
Bodwell, C. E. (1971) *Contractile proteins and muscle*, ed. K. Laki (Maryland: Bethesda).
Chavancy, G., Daillie, J. and Garel, J. P. (1971) *Biochimie*, **53,** 1187.
Daikee, E.(1976) *Biol. Neonate*, **28,** 27.
Dure, L. S. (1973) *Molecular techniques and approaches in developmental biology*, ed. M. J. Chrispeels (New York: Wiley Interscience) p. 199.
Elska, A., Matsuka, G., Matiash, U., Nasarenko, I. and Semenova, N. (l971) *Biochim. Biophys. Acta,* **247,** 430.
Frazer, J. M. and Yang, W. K. (1972) *Arch. Biochem. Biophys.,* **153,** 610.
Garel, J. P., Mandel, P., Chavancy, G. and Daillie, J. (1970) *FEBS Lett.*, **7,** 327.
Garel, J. P., Mandel, P., Chavancy, G. and Daillie, J. (1971) *FEBS Lett*, **12,** 249.
Hoffman, J. L. (1972) *Fed. Proc. Fed. Am. Soc. Exp. Biol*., **31,** 866.
Hoffman, J. L. and McCoy, N. T. (1974) *Nature (London)*, **249,** 558.
Klee, H. J., Pietro, D. D. and Fournier, M. S. (1978) *J. Biol. Chem*., **253,** 8074.
Laki, K. (1971) *Contractile proteins and muscle,* (Maryland: Bethesda).
Lanks, K. W. and Weinstein, I. B. (1970) *Biochem. Biophys. Res. Commun*., **40,** 708.
Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem*., **193,** 265.
Maepaa, P. H. and Ahonen, J. (1972) *Biochem. Biophys. Res. Commun*., **49,** 179.
Mans, R. J. and Novelli, G. D. (1961) *Arch. Biochem. Biophys*., **94,** 48.
Mays, L. L., Lawrence, A. E. and Rose, W. (1978) *Fed. Proc. Fed. Am. Soc. Exp. Biol*., **37,** 454.
Mettler, I. J. and Romani, R. (1976) *Phytochemistry*, **15,** 25.
Palatnik, C. M. and Katz, E. R. (1977) *J. Biol. Chem.* **252,** 694.
Portugal, F. H. (1972a) *Mech. Age. Dev*., **1,** 3.
Portugal, F. H, (1972b) *Mech. Age. Dev*., **1,** 23.
Reitz, M. S. and Sanadi, D. R. (1972) *Exp. Gerontol*, **7,** 119.

Schneider, W. C. (1957) *Methods Enzymol*, **3,** 680.
Sherton, C. C. and Wool, I. G. (1974) *Methods Enzymol*, **30,** 506.
Shugart, L. (1972) *Exp. Gerontol*., **7,** 251.
Smith, D. W. and McNamara, A. L. (1972) *Biochim. Biophys. Acta*, **269,** 67.
Yang, W. K. and Novelli, G. D. (197 1) *Methods Enzymol*, **20,** 45.