Drugs and Aging

Edited by Dieter Platt

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Preface

Experimental gerontological research is necessary to obtain optimal information and thus ensure proper drug therapy for the elderly. Most older persons acquire multiple diseases, first of all chronic diseases. They involve complex problems of a physical, social, and psychological nature. The multimorbidity of the elderly raises many questions in drug therapy. By contrast with our extensive knowledge of pharmacokinetics and pharmacodynamics in younger age groups, few facts are available in respect of the elderly. A variety of factors may influence drug therapy. Physiological and pathological age-related changes of molecules, cells, organs, and the total organism may interact to enhance or inhibit drug therapy in higher age groups. It is well known that elderly patients are overmedicated and therefore the incidence of adverse drug reactions increases with age.

Elderly patients with multimorbidity often have a diminished body clearance of drugs; age-related changes of the kidney seems to be one of the most important factors in this regard. Far less important than the elimination of drugs through the kidney is their excretion in the bile. So far results have disclosed that oxidative steps in drug clearance are more likely to be disturbed than phase II reactions. Furthermore, changes in distribution volume and age-related alterations in receptor sensitivity also influence the clearance of drugs in the elderly.

The contributions compiled in this book cover drug actions and interactions at the molecular, cellular, organ, and organism level. They are based on the lectures and discussions of the 2nd International Erlangen-Nuremberg Symposium on Experimental Gerontology (November 28–December 1, 1984) in which many distinguished scientists in the fields of biology, biochemistry, pharmacology, pathology, and medicine participated. This book will expand the knowledge of drug therapy in the elderly and will provide a firm foundation for future work on clinical and experimental pharmacology in the aged.

I wish to thank Albert-Roussell, Wiesbaden, Boehringer Mannheim GmbH, Mannheim, Hoechst AG, Frankfurt, ICI-Pharma, Plankstadt, Lipha Arzneimittel GmbH, Essen, Luitpold-Werk, Munich, Dr. Madaus and Co., Cologne, Röhm Pharma GmbH, Darmstadt, Schaper & Brümmer, Salzgitter, and Zyma GmbH, Munich, for the financial assistance they have provided for the meeting in Nuremberg.

Erlangen-Nuremberg — DIETER PLATT

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Age-Related Changes of Transcription and RNA Processing

Z. A. MEDVEDEV [1]

Introduction

Among the three main groups of biological macromolecules uniquely linked by transfer and expression of genetic information: (DNA – RNA – Proteins), the RNA stage is the least studied for possible age-related changes. There have been many recent reviews about age-related changes of DNA and proteins, or DNA–protein complexes in chromatin. Recently however, there has been only one review on the role of RNA and RNA metabolism in aging (Rothstein 1982), and a brief supplement to it (Eichhorn 1983) which summarizes the available experimental data up to 1980 on the age-related changes of the synthesis of different types of RNA (rRNA, mRNA, and tRNA). The authors have not linked factual information with several relevant theories of aging [genetic program theory, codon-restriction theory, loss of rRNA genes theory, loss of gene repression theory (dysdifferentiation), general error theory, and others which cannot be proved without special study of transcription and RNA] simply because the information about the age-related changes of RNA is very limited and too general. There have recently been two reviews on the age-related changes of chromatin (Thakur 1984; Medvedev 1984), which also included attempts to cover the connected problems of age-related changes of DNA and the pattern of transcription. Here we would like to select the role of RNA in the aging process for more detailed analysis. The amount of knowledge in this field has not increased dramatically during the last 3–4 years, although there have been many quite interesting studies of changes of RNA in aging tissues which could be added to the list of studies covered by previous reviews. However, we would like to use a different approach which may prove useful. Rothstein (1982) and Eichhorn (1983) have discussed the problem of age-related changes of RNA directly and tried to show what is really known about RNA and transcription in old tissues and cells. We want to look at the same problem from current knowledge of RNA synthesis in general and to expose some gaps in age-related studies of RNA. It is often important to describe not only the information which is *known,* but also the potential in widening a research net. There were many authors in the 1950s and 1960s who studied the age-related changes of the content of different RNAs in tissues and the activity of RNA synthesis and were convinced that the synthesis of RNA is a one-step process which needs only one RNA polymerase. The discovery of the "multi-cistrone" RNA

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(hnRNA), RNA processing, splicing, introns, post-transcriptional modification, separate polymerases for ribosomal, transfer, and messenger RNAs, polyadenylation of mRNA, the selectivity of nuclear–cytoplasmic transfer of RNA, and some other intermediate processes which divide initial transcripts from their complex function in protein synthesis have rapidly made our knowledge on the role of RNA in aging very obsolete. Therefore, an attempt to compare what is known about RNA synthesis and about RNA aging could still be useful, even if it does not add much to existing knowledge about the role of RNA in the aging process, already considered by Rothstein (1982). We also speculate about the possible role of RNA processing in the elimination of the alterations of DNA which cannot be removed by the DNA repair systems.

Taking into account the special interest of this symposium in the problem of drug effects related to the molecular and cellular level of aging, we shall make some comments about the attempts to use RNA, ribonucleotides, and some RNA synthesis stimulating drugs for different geriatric treatments.

Age-Related Changes of RNA Content and RNA Synthesis

Most studies show the age-related decrease of RNA content and RNA synthesis in tissues and per diploid cell. (For review see: Medvedev 1964; Berdyshev 1970; Rothstein 1982). Some results on the incorporation of labeled nucleotides into nuclei which originally indicated the possibility of a steady increase of RNA turnover in old tissues (Wulff et al. 1962, 1964, 1966) and stimulated the formulation, by the authors, of a theory of increased synthesis of nonfunctional defective RNA (Wulff et al. 1962; Wulff 1966) were later shown to be artifacts. The authors used labeled nucleotide precursors with high specific activity and tested their incorporation after short intervals. They did not take into consideration the sharp reduction of the free nucleotide pool in aging tissues which made the labeled precursors much less diluted (before incorporation) (Bucher and Swarfield 1966; Davi et al. 1966). Current studies of the age changes of the pool of free nucleotide monophospates and nucleotide bases in liver nuclei (Bolla and Miller 1980) have found an almost eight- to tenfold reduction in their concentration.

The age-related decrease of RNA synthesis (usually in liver and muscles from rats) has been shown with different RNA precursors – [^{32}P] (Shereshevskaya 1963; Nikitin and Shereshevskaya 1962), [^{3}H] uridine (Soriero and Talbert 1975), and [^{14}C] uracil (Kanungo et al. 1970) and by other methods (Schneider and Shorr 1975; Macieira-Coeho and Loria 1976). Some authors have incubated isolated nuclei in vitro with labeled nucleotides (Devi et al. 1966; Mainwaring 1968; Britton et al. 1972; Castle et al. 1978) and confirmed the same tendency. However, it would be more interesting to find out how this general conclusion about the age-related reduction of RNA synthesis and nucleotide metabolism (size of nucleotide pool) is reflected in the synthesis and turnover rates of functionally specific groups of RNA.

Ribosomal RNA

The largest proportion of cellular RNA is represented by ribosomal RNA (rRNA). rRNAs are the final gene products and they make an important structural element of several million ribosomes in each cell. The proliferating tissues and particularly developing tissues need a very active synthesis of rRNAs. The proper supply of rRNA is possible only because each cell normally contains multiple, highly redundant copies of ribosomal genes. The level of this redundancy is species specific. Human cells contain about 200 rRNA genes per haploid genome, but this figure could reach 1000 or higher in some amphibian species. The high level of the redundancy of rDNA is necessary for critical development stages. Adult tissues, and particularly those which have no mitotic activity, do not need so many ribosomal genes. It is not yet clearly established whether the rate of transcription of ribosomal genes is sharply reduced in differentiated cells, or whether most rRNA genes remain idle and inactive while a few continue to be transcribed at a high rate. The ribosomal genes are transcribed by RNA polymerase I. The original transcript is a precursor (45S RNA) and its post-transcriptional processing includes a cleavage which produces one copy each of 28S rRNA, 18S rRNA, and 5.8S rRNA, which are assembled into ribosomes in a special structural unit – the nucleolus. The accuracy of all these steps is important because the functional ribosomes have a significant influence on the fidelity of translation.

Age-related studies of the synthesis of rRNA were stimulated by a report by Johnson and Strehler (1972) and Johnson et al. (1972), who found a substantial decrease of ribosomal genes (rDNA) in several tissues of the aging beagle. The loss of rDNA was tissue specific, rapid in brain, heart, and muscles, and less rapid in spleen, kidney, and liver. The authors suggested that such a decrease may reduce the synthesis of rRNA to a level insufficient to support the functions of all the ribosomes necessary for cellular functions. However, they did not compare the actual rate of synthesis of rRNA, tRNA, and mRNA to find any disproportion in the rate of synthesis. It has, therefore, remained unclear whether the loss of rDNA (estimated by the molecular hybridization) could cause some kind of "ribosomal deficiency" or whether remaining rRNA genes are still able to support the formation of ribosomes, which is apparently very slow in differentiated cells.

The possible loss of rRNA genes in aging tissues was later shown for mouse brain, spleen, and kidney tissues (Gaubatz and Cutler 1978), while loss of rDNA was shown in human myocardium and cerebral cortex (Strehler 1979; Strehler and Chang 1979). Shmookler-Reis and Goldstein (1980) found a loss of reiterated sequences during serial passage of human diploid fibroblasts. Buys et al. (1979) studied the age-dependent changes of transcription of ribosomal genes in human lymphocytes and fibroblasts. They were able to register a rather extensive loss of ribosomal RNA gene activity. However, the authors did not consider it a sign of abnormality – they expected from the results of some other tests to show that human cells may easily survive and withstand the loss of a significant part of their highly repetitive ribosomal RNA genes. A decrease of the synthesis of ribosomal RNA in hearts of aging rats was found by Yavich et al. (1978). An age-correlated loss of ribosomal RNA synthesizing capacity was shown for macronucleus in

Paramecium (Heifetz and Smith-Sonnenhorn 1981). However, in a recent study (Peterson et al. 1984) no differences in rRNA gene copy number were found during in vivo aging of mouse myocytes or in vitro aging of the W1-38 line of human fibroblasts. The authors suggest that the loss of rDNA is not a ubiquitous feature of aging of mammalian cells. In mouse heart cells the number of 28S rRNA genes per haploid genome was 90 in young, 102-107 in 15- to 25-month-old, and 93 in 39-month-old animals, with a standard deviation of about 5–6. The authors applied a different method of DNA purification and hybridization, using more extensive deproteinization procedures, and suggested that the previous reports showing a sharp decrease of rDNA were influenced by the presence of tightly DNA bound proteins in older cells which have reduced the hybridization capacity of DNA. Attempts to study age-related changes of processing of rRNAs are still absent. Indirect evidence was reported by Mori et al. (1978), who found a decrease in the cytoplasmic ratio of 28S and 18S ribosomal RNA in mouse liver and brain tissues. In polysomal RNA both 28S and 18S RNA were present in equimolar amounts. However, in cytoplasm of older tissues there was some molar excess of 18S RNA. 18S and 28S ribsomal RNAs are transcribed from the same cistron. The excess of 18S RNA can be related either to slower transportation of larger 28S RNA from nucleus to cytoplasm or a specific higher level of degradation of 28S RNA.

Transfer RNA

Strehler (Strehler et al. 1967, 1971; Strehler 1977) proposed a theory which considered some changes in the pattern of tRNAs and isoaccepting rRNAs as a cause of developmental alterations and their continuation into age-related alterations as well. The authors were able to demonstrate apparent changes of the pattern of isoaccepting tRNAs and tRNA synthetases during morphogenetic "aging" of soybean cotyledon (Bick and Strehler 1971, 1972) and plant leaves (Wright et al. 1972; Andron and Strehler 1973). However, in plants and particularly in individual plant organs the distinction between aging and genetically programmed development is difficult. Developmental aging is a special problem of gerontology and it is more relevant to plants and some short-lived animal species with a single reproduction cycle. In most animal species, and particularly in mammalian species, development and aging are well separated in time, so that the character of changes of tRNAs which are specific for development and which are relevant to real postmaturation senescence can be studied separately.

Age-related changes of the pattern of isoaccepting forms of tRNA have been reported for nematode (Reitz and Sanadi 1972) and *Drosophila* (Hosbach and Kubli 1979; Owenby et al. 1979). The appearance of some deficient, inactive molecules of tRNAs in aging *Drosophila* has also been reported (Hosbach and Kubli 1979 b). Transfer RNAs have many modified nucleotides which are necessary for their function and complex secondary structure. In aging cells some additional modifications have been observed, like the change of guanine to queuine (Singhal and Kopper 1981), or the reduction of methylation (Mays et al. 1979), which could lead to higher infidelity of translation.

There have been few attempts to study age changes of tRNA in mammalian tissues. In most cases the changes were minor and in one or two types of tRNA only (Lawrence et al. 1979). Rothstein (1982), who reviewed the literature up to 1980, suggested that for some more definite conclusions we "simply have to wait for more data to become available" (p. 187).

New data are still slow in coming. It was shown that in mouse kidney and heart the content of tRNA and the rate of tRNA synthesis decline with age (Neumeister and Webster 1981). Cook and Buetow (1982), who studied the pattern of tRNAs in the liver of the senescent female Wistar rat, did not, however, find any visible changes related to aging. No significant age-related differences were found in the extent of aminoacylation of the liver cytoplasmic tRNA population, the total tRNA synthetase activity, the rate of aminoacylation of individual tRNAs, or the overall complement of tRNA species as detected by two-dimensional gel electrophoresis. However, some undetected changes were still possible because the tRNA system (tRNAs plus aminoacyl tRNA synthetases) isolated from livers of old animals were less capable of supporting in vitro cell-free protein synthesis than were the same fractions isolated from adult (10- to 13-months-old) animals. This may indicate that some types of tRNA which show the same electrophoretic mobility are "defective," and these defects may include base modifications of a different kind. The authors have tested individual tRNAs in homologous and heterologous assays. They found that in heterologous assays adult tRNA synthetases isolated from adult animals were significantly more active than senescent synthetases when charging isoleucine, methionine, phenylalanine, proline, and glutamic acid, and less active when charging alanine, aspartic acid, and serine. There were some other differences for in vitro synthesis which were not visible in vivo.

Transfer RNA genes contain introns, but this is not the rule for all tRNA species. Only one-fifth of yeast tRNA genes contain introns (Johnson and Abelson 1983). This means that the processing pattern is different for individual tRNAs; some do have splicing, while others do not need it. It is probable that the more complex the processing of transcribed RNA, the more alteration-prone would be the functional molecules. If one could show that these tRNAs which need to be processed are also more "age-changeable," this may indicate the possibility that the error rates vary for each type of RNA macromolecule.

Messenger RNA

We discussed previously that the synthesis of rRNA declines with age. Some earlier studies (Detwiler and Draper 1962) indicated that the synthesis of messenger RNA may decline even more rapidly in aging tissues. However, if for rRNAs and for tRNAs it was possible to study some changes of individual molecular species (there are four main rRNAs and probably about 100 different species and isoforms of tRNAs), the same task was too difficult for mRNAs. Each cell has several thousand different messenger RNAs, some of them for minor protein represented by a few RNA molecules, others for major proteins represented by several million mRNAs. Despite this high heterogeneity, only about 5%–7% of cytoplasmic RNAs belong to the mRNA group; 70%–80% belong to rRNA, and 10% –15% to tRNA, both the latter groups having longer-lived molecules.

Messenger RNA is transcribed by RNA polymerase II. The structure of mRNA genes reflects the evolutionary history of different proteins. Most acient proteins, like histones or protamines, are coded for by reiterated genes assembled into special clusters. These genes normally have no introns and their transcripts have simple processing [without forming a poly(A) tail]. Genes of more recent origin, like genes for immunoglobulins, have very complex structures with many introns. Their RNA transcripts have to pass through extremely sophisticated processing before they finally form mRNAs. For most proteins the processing of their mRNAs includes the formation of precursor (initial transcript, or hnRNA), splicing, capping (from 5′ end – formation of a special structure which is necessary for later binding of mRNA to ribosome), and polyadenylation of their 3′ ends. Polyadenylation (by poly-A-polymerase) adds 50 to 250 residues of adenylic acid. (For review see Brawerman 1976; Edmonds and Winters 1976.)

If hnRNA has many introns the splicing may need to pass through several stages. Information transfer and biological reactions are never perfect and some errors are possible at every stage. *It is therefore possible to suggest that those mRNAs which have the simplest processing accumulate fewer errors, while mRNAs with complex and multistaged processing would transfer more errors into translation.* It is possible to test this hypothesis by showing, for example, that the transfer of information from DNA to histones is more accurate than the transfer of information from DNA to immunoglobulins or other proteins coded by genes with many introns. However, the actual state of the age-related studies of mRNA is still very far from the possible estimation of the accuracy of synthesis of individual messengers.

Attempts to study qualitative age changes of messenger RNA were started only recently. They became possible with the development of some methods which allow the separation of polyadenylated forms of RNA. The first to apply these methods to the study of poly(A) RNA from young and old tissues were Müller et al. (1979, 1980), who measured the synthesis of polyadenylated RNA in oviducts from adult and senescent quails and by hepatocytes isolated from rats of different ages. The authors found that in senescent animals the poly(A) stretch of mRNA is shorter than in adult animals. The rate of poly(A) RNA synthesis by hepatocytes decreased by 68% between 6 and 30 months of age. The rate of total RNA synthesis also decreased, mostly until 19 months of age. Between 19 and 30 months of age the decrease in synthesis of polyadenylated RNA continued (about 40%), while the decrease in total RNA synthesis (mainly rRNA and tRNA) was not significant. The decrease in poly(A) synthesis is most probably related to the decrease in the transcription rate. However, a decrease in the processing rate is also possible. Later Richardson et al. (1982) confirmed that the decline in the synthesis of rRNA and tRNA in aging hepatocytes was less rapid than the decrease in the synthesis of poly(A) mRNA.

The polyadenylated segment of mRNA, or poly(A), has important regulatory functions. It is relevant for the transport of mRNA through the nuclear membrane and (according to some evidence) for the regulation of the cytoplasmic functional life span of mRNA. Synthesis and degradation of poly(A) includes several enzymes, both anabolic and catabolic. Müller and collaborators were not able to analyze the individual species of mRNA which control the synthesis of

specific proteins. However, they carried out a complex experiment to ascertain the effect of aging on poly(A) metabolism of mRNA in two groups of female quails: mature (250–320 days old) and older animals (3–3.5 years old) (Arendes et al. 1980; Bernd et al. 1982). They found that the average size of the poly(A) segment of mRNA decreases with age. In mature animals the average poly(A) segment was 120–180 AMP units in the oviduct and 100–110 in heart and liver. In older animals the poly(A) chains were about 70 AMP units long.

The decrease in the size of the poly(A) segment with age could be responsible for the sharp decline of mRNA synthesis. Because the methods of isolation of mRNA are now based on the binding of its polyadenylated terminus, the yields of mRNA isolated from older tissues could be lower simply because of shorter poly(A) segments. The results showing a sharp decrease in poly(A) mRNA synthesis related to aging in brain and liver cells were confirmed by several authors (Semesei et al. 1982; Dilella et al. 1982). Katsarada et al. (1982) found an age-related decline in the synthesis of specific mRNA liver malic enzyme (by measuring the substrate induction).

Chatterjee et al. (1981) were the first group who tried to find qualitative age-related changes of mRNA coding for specific hepatic proteins. Such changes could be deduced from the alterations of the pattern of synthesized proteins. However, the authors tried to approach the same problem in a more direct way, by the analysis of translational products of poly(A) mRNA isolated from liver of animals of different ages. The actual translation was carried out in vitro. Liver mRNA was mixed with the rabbit reticulocyte lysate. Newly synthesized proteins were labeled with ^{35}Smethionine and identified by autoradiography. The authors found that three proteins show evident age-related variations and they called these proteins "senescence marker proteins," or SMPs. One of these proteins was characterized as globulin (SMP-3). Aging was connected with the disappearance of mRNA for SMP-1 and SMP-23. The authors suggested that their results were consistent with the concept of genetically programmed aging.

The results of this particular work should, however, be treated with some reservations because of the very low activity of the mRNA in this in vitro system. Only four or five bands of ^{35}Smethionine-labeled proteins were visible in the areas of electrophoresis between molecular weights 12,000 and 43,000. In normal liver cytoplasm there are several dozen proteins which can be identified by in vivo synthesis with the same label and by one-dimensional SDS-gel electrophoresis. Anzai et al. (1983), who have used an improved method of in vitro synthesis and two-dimensional electrophoresis, carried out tests on poly(A) RNA translational activity with ^{35}Smethionine isolated from liver,kidney, and brain of mice of different ages. They were able to identify by autoradiography about 300 spots and most of them were highly reproducible. The authors registered an apparent tissue specificity of the pattern of spots, while the age-related variations were not significant. In kidney and brain none of these spots consistently depended on age. Only in livers did the authors observe one translation product (with a molecular weight of about 30,000) which could be associated with senescent mice. The authors concluded that most of the major genes which are expressed in the tissues of young mice (3.5–14.4 months old) and adult mice are also expressed in senescent animals (22–29 months old). The age-related protein from liver was not identified and the

authors did not find this protein when they tried to analyze a liver extract of senescent mice.

This approach, like all other attempts to use to poly(A) segment for isolation of mRNA, can miss some changes of mRNA which are expressed in the reduction of poly(A) segments reported by Müller et al. (1979, 1980). It is obvious that not only the existence of mRNAs without a poly(A) segment may influence the results but also the presence of mRNAs with a short poly(A) sequence. About 30%–40% of the total mRNA in mouse liver is reported (Moffett and Doule 1981) to have poly(A) too short to bind to oligo(dT) columns which are used for isolation of poly(A) RNA. Schröder et al. (1983) have recently developed a method which is able to isolate the shorter poly(A) sequences, even oligo(A) segments down to a chain length of four nucleotide units. In nondividing mouse lymphoma cells the amount of $A_2^-{}_6$ sequence labeled by ^3H was at the level of 8% of the total amount of poly(A) sequences. This means a much higher percentage of mRNAs with short poly(A) segments. The authors applied the new method to their earlier studied experimental model of young and old female quails (Müller et al. 1979) and found that the amount of low molecular weight oligo(A) fragments also gradually decreases during aging of animals. One may assume that if the length of poly(A) segments shortens with age and the number of oligo(A) segments also decreases with age, the aging should correlate with an increase of mRNAs which are not polyadenylated.

Khasigov et al. (1983), who recently studied the rates of polyadenylation of mRNA in relation to aging, also tried to isolate poly(A) RNA and oligo(A) RNA separately. In addition they isolated nuclear precursors of mRNA from livers and brain of young and adult rats (the oldest animals were 540 days old). The rate of polyadenylation decreased with age, whereas the half-life time of precursors of mRNA increased. This means slower processing of RNA in older nuclei.

A very specific test to study the age-related changes of the pattern of mRNA was tried by Ono and Cutler (1978). They found that the amounts of endogenous leukemia virus-related and globin-related mRNA increase in the brain and liver of mice with age. The authors regard this as a possible derepression of some genes in older animals and the "leaky expression" of mRNAs which are not normally required for certain types of differentiation. These results were presented to support the theory of cellular aging as a kind of dysdifferentiation process (Cutler 1982). However, the possible appearance of "leaky mRNAs" in aging cells is only one of very many possible changes of different groups of RNA which may disturb normal function of tissues (see Table 1).

Grady and Campbell (1981) did not find changes in the RNA complexity when they studied poly(A) RNA in rat tissues. However, they have analyzed tissues of only 2- and 10-month-old animals. Yavich (1981) did not find the changes in the poly(A) mRNA pattern in myocardium from rats between 3 and 24 months of age.

If aging does correlate with the shortening of the poly(A) tail, which seems to be the case, then the changes of the mRNA population in old tissues could be selectively missed during the isolation procedure. The method selectively isolates less changed mRNA population. There are at least 10,000 different mRNA species in mouse liver (Hastie and Bishop 1976), and a method which can detect

quantitative and qualitative age-related changes is still not available. It is also known that the method of in vitro synthesis only shows translation products of relatively abundant mRNA species. At the same time mRNAs of low or medium abundance are the most likely messengers for enzymes and proteins with regulatory functions – these may be the most important for age-related studies, and probably the most vulnerable to alterations. The highly abundant species of mRNA are more likely to be coded for by reiterated genes or by families of closely related genes. It has been suggested (Medvedev 1972, 1983; Cutler 1974) that gene reiteration and all the other forms of redundancy of information may act as an age-protective mechanism.

RNA Turnover

rRNA and tRNA

All forms of ribosomal RNA are quite stable; different species of tRNA have shorter half-lives, while mRNAs in most cells are very short lived. The high turnover rate of mRNAs made it possible in the late 1950s to identify this RNA as "informational" by "pulse labeling." In the 1950s and 1960s, age-related studies of turnover rates of RNA were usually carried out without fractionation of RNA into different functional groups. The first experiments in which the half-lives of RNA were measured in young and old rats for rRNA and tRNA separately were carried out by Menzies et al. (1972). The half-lives of rRNA were tissue specific and varied between 5.9 and 8.9 days in liver, spleen, kidney, and other tissues. No age-associated differences were observed. tRNA fraction was apparently very heterogeneous and had longer- and shorter-lived fractions (variations of half-lives between 1 and 11 days). The authors suggested the possible presence of abnormal tRNAs and rRNAs and their preferential degradation, but they were not able to confirm this with experimental data. In later work (Menzies and Aguilar 1973) the measurement of the decay of radioactivity of RNA was extended (up to 90 days) after a single injection of ^{14}Corotic acid. The authors found the existence of very long-lived forms of rRNA (with half-lives up to 29 days), but no clear age-related differences.

mRNA

The first study in which age-related changes of half-lives of messenger RNA were investigated was carried out by Wattiax et al. (1971). The new synthesis of mRNA was inhibited by actinomycin D, and the authors studied the functional lives of pre-existing mRNAs (by their ability to support the protein synthesis) in the thorax and ovary of *Drosophila melanogaster*. It was found that the half-lives of mRNA was shorter in older flies.

There is, as yet, no satisfactory explanation why some proteins are translated from the short-lived mRNAs and others from long-lived mRNAs. The necessity of the long-lived mRNA for terminal differentiation in reticulocytes (Gorsky et al. 1974; Nokin et al. 1976) is understandable, since this allows the reticulocytes to continue the synthesis of hemoglobin after the synthesis of most other proteins