Changes in the Ribonucleic Acid Metabolism of Aging Mouse Tissues with Particular Reference to the Prostate Gland

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1. The synthesis of RNA in purified nuclei of tissues from aged mice is substantially diminished. 2. As judged by the recovery of RNA in 105 000g supernatants of tissue homogenates, the synthesis of ribosomal RNA may be particularly affected. 3. The decrease in RNA synthesis may be due to changes in the composition of the nuclear-associated protein with age, which result in changes in the priming ability of tissue chromatin. 4. Aging is accompanied by marked changes in the sedimentation profiles of ribonucleoprotein particles. These can be attributed to an age-associated depletion in messenger RNA or to an increased susceptibility to enzymic disaggregation.

The correlation between aging and the incidence of human prostatic cancer is established beyond all doubt (e.g. see Moore, 1935; Franks, 1956). The experimental investigation of this correlation is perhaps best performed on inbred animals, since their maintenance in a controlled environment may result in a more uniform aging population. The C57 mouse is a suitable animal for studies on the prostate, for many of the histological changes occurring in old age are similar to those found in man (Franks, 1959). Some of the biochemical changes occurring in the aging mouse prostate include a decrease in the total protein content and changes in the specific activities of many enzymes (Mainwaring, 1967, 1968). In the absence of gross qualitative differences in isoenzyme patterns, a possible explanation of these findings is that the synthesis of protein, including enzymes, is impaired in old age. Since protein synthesis is ultimately dependent upon RNA, changes in the RNA metabolism of the aging prostate were investigated. Other tissues were included in the study for comparison. No major differences between the RNA contents of young and old tissues have been reported (Barrows, Yiengst & Schock, 1958; Quarto di Palo, Mombelli & Gastaldi, 1964), but such data give no clear indication of the state of RNA synthesis, particularly of those RNA species with a high rate of turnover. Particular attention was given to changes in nuclei from aged animals that could influence DNA transcription. Medvedev (1964) and Hahn (1964) suggested that aging may be due to structural changes in the genome that ultimately lead to anomalies or deletions in messenger RNA synthesis. This suggestion was studied

indirectly by the examination of the RNP* populations in aged tissues, for it is known that messenger RNA binds ribosomes into polymeric aggregates (polysomes) (Warner, Rich & Hall, 1962). Further, since the polysomes are particularly active in protein synthesis (Wettstein, Staehelin & Noll, 1963), changes in their proportion of the total RNP population would be expected to affect protein synthesis.

MATERIALS AND METHODS

Chemicals. ATP, CTP, GTP, UTP, highly polymerized calf thymus DNA, crystalline bovine serum albumin, crystalline deoxyribonuclease (type DN-100), tris base (99.5% pure) and spray-dried *Micrococcus lysodeikticus* cells were purchased from Sigma (London) Chemical Co., London, S.W. 6. [³H]UTP (tetralithium salt; 2.24 Ci/mole) was obtained from Schwarz BioResearch Inc., Orangeburg, N.Y., U.S.A. Crystalline ribonuclease was obtained from British Drug Houses Ltd., Poole, Dorset. Pronase (grade B) was supplied by California Biochemical Corp., Los Angeles, Calif., U.S.A. Chemicals were of A.R. grade, and were generally used without further purification. However, diphenylamine was recrystallized from 70% (v/v) ethanol, and 3,5-diaminobenzoic acid was purified with charcoal and repeatedly crystallized as the dihydrochloride salt before use. RNA polymerase (EC 2.7.7.6) was isolated from *M. lysodeikticus* by procedure A of Nakamoto, Fox & Weiss (1964) to step V, and was stored in 50% (w/v) glycerol at -15°. DNA was isolated from the prostate glands of 6-month-old mice by the procedure of Kay, Simmons & Dounce (1952) and further purified by incubation with deoxyribonuclease-free enzymes, first ribonuclease and then Pronase. Solutions of ribonuclease were heated at 90° for

*Abbreviation: RNP, ribonucleoprotein.

10 min. before use, and deoxyribonuclease contamination in solutions of Pronase was removed by incubation at 37° for 2 hr. Rat liver RNA was prepared in the conditions of McIndoe & Munro (1967), and yeast RNA was isolated by the method of Crestfield, Smith & Allen (1955).

Chemical analyses. The method of Lowry, Rosebrough, Farr & Randall (1951) was used for the determination of protein, with bovine serum albumin as reference. Tissue samples for nucleic acid analysis were precipitated in the cold in 0.4N-HClO₄ and washed twice with cold 0.2N-HClO₄. RNA was selectively extracted from the residue into KOH and measured as described by Fleck & Munro (1962). DNA was extracted from the acid-insoluble material in 0.5N-HClO₄ at 70° and determined by the method of Burton (1956), with calf thymus DNA as standard. Where material was scarce, DNA was determined by the fluorimetric procedure devised by Kissane & Robins (1958).

Counting of radioactivity. ³H was counted in a Packard Tri-Carb model 3003 scintillation spectrometer at an efficiency of 40–42%. Quenching was calculated by the channels-ratio method (Baillie, 1960). Phosphor was prepared by dissolving 100 g. of naphthalene and 3 g. of 2,5-diphenyloxazole in 385 ml. of xylene, 385 ml. of dioxan and 230 ml. of ethanol.

Animals. Virgin C57 black-and-tan mice were used, and the terms 'young' and 'old' refer to animals of 6 and 30 months of age respectively. Animals were killed by cervical dislocation and the tissues were rinsed twice in the appropriate ice-cold medium for the subsequent isolation of various subcellular particles.

Isolation of subcellular particles. Homogenization of tissues was performed at 0–4° with a motor-driven Potter-Elvehjem homogenizer with a Teflon pestle. All subsequent procedures were carried out as near to 0° as possible.

(a) **Nuclei.** Tissues were homogenized in 0.25 M-sucrose containing 1 mM-MgCl₂ and the homogenates were filtered through 100-gauge stainless-steel grids. The impure nuclei were collected by centrifugation at 700g for 10 min. and washed twice in sucrose-MgCl₂ solution. The washed nuclei were resuspended in 0.5 ml. of sucrose-MgCl₂ and layered over a discontinuous gradient composed of 2.0 ml. of 2.0 M-sucrose and 2.0 ml. of 1.6 M-sucrose (Allfrey, Littau & Mirsky, 1964). After centrifugation at 50 000g_{av.} for 30 min. in a swing-out rotor, the pellet of purified nuclei was resuspended in 0.5 ml. of 0.25 M-sucrose-1 mM-MgCl₂. The nuclei were always examined by phase-contrast microscopy to establish that the preparations were essentially free of cytoplasmic contaminants and cell debris. In the preparation of samples for photography, nuclear smears were fixed in 10% (v/v) formalin in neutral phosphate buffer for 1 hr., rinsed in water and mounted in 1% (w/v) polyvinylpyrrolidone. To check the biochemical purity of the nuclei, samples were taken at all stages of the preparations and analysed for DNA and the 'marker' enzymes cytochrome oxidase, glucose 6-phosphatase and glucose 6-phosphate dehydrogenase. The assay conditions were described by Mainwaring (1967).

(b) **RNP particles.** Method II of Liao & Williams-Ashman (1962) was used for the isolation of RNP particles, but with the modification that deoxycholate was replaced by Triton X-100 (Hunter & Korner, 1966), added at a final concentration of 1% (w/v), for the solubilization of endoplasmic reticulum. The RNP particles were gently resuspended in 10 mM-tris-HCl buffer, pH 7.8, containing 10 mM-MgCl₂ and

50 mM-KCl (medium A) in a loose-fitting TenBroeck homogenizer. The proportion of non-sedimentable RNA was determined by a comparison of the RNA contents in the initial homogenate and in the 105 000g supernatant fraction. In certain cases the samples of 105 000g supernatant were dialysed for 72 hr. against medium A or subjected to gel filtration on columns of Sephadex G-25 equilibrated with medium A, before RNA analysis. Electrophoretic examination of the RNA present in the 105 000g supernatant was conducted in the conditions of Richards, Coll & Gratzer (1965). Samples of the initial homogenates were also analysed for ribonuclease activity. Replicate samples of homogenate (25–50 μl.) were incubated at 37° with 50 μl. of 0.1 M-tris-HCl buffer, pH 7.5, and 50 μl. (500 μg.) of yeast RNA. At timed intervals samples were cooled in ice and treated with 2.5 ml. of ice-cold ethanol-0.2 N-HClO₄ (3:2, v/v). After centrifugation in the cold, the extinction of the supernatants was measured at 260 mμ. The extinctions were corrected for zero-time controls in which precipitant was added to the homogenate before the addition of substrate; these controls were not incubated. By using the extinction coefficient of hydrolysed RNA given by Munro & Fleck (1966) the ribonuclease activity was calculated from the initial rate of hydrolysis.

(c) **Chromatin.** This was prepared by the method of Paul & Gilmour (1966). The final preparations were gently dissolved in water with a loose-fitting homogenizer. There was a noticeable difference in the appearance of the chromatins isolated from various tissues; that from prostate and lung tissue was gel-like and filamentous, whereas liver chromatin, despite excessive washing, was amorphous.

Assay of RNA synthesis. (a) **Nuclear RNA polymerase.** The assay medium (total volume 0.25 ml.) contained: 50 μmoles of tris-HCl buffer, pH 8.1; 2.5 μmoles of MgCl₂; 2.5 μmoles of β-mercaptoethanol; 0.2 μmole each of ATP, CTP and GTP; 5 μmoles of UTP and 0.2 μCi of [³H]UTP. The reaction vessels were thoroughly cooled in an ice bath, and 0.25 ml. of nuclear suspension, equivalent to 50–125 μg. of DNA, was added. After 15 min. incubation at 37° the tubes were returned to the ice bath and 50 μl. of 50% (w/v) trichloroacetic acid was added. After 10 min. the precipitates were collected in the cold on Millipore filters (code no. PHWP 02500), previously soaked in 5% (w/v) trichloroacetic acid and washed three times with 5 ml. of 5% trichloroacetic acid. The membranes were placed in 10 ml. of phosphor and counted in a scintillation spectrometer. Controls in which the precipitant was added before the nuclei were run for each preparation. Counts were corrected for controls and background, and the results were expressed as μmoles of UTP incorporated/15 min./100 μg. of DNA. This method of assay gave essentially similar results to the more elaborate procedure in which the ³H-labelled RNA was selectively extracted into KOH at 37°. The inclusion of 20 μg. of ribonuclease or deoxyribonuclease decreased the incorporation by 95–98%.

(b) **Chromatin-primed synthesis.** The priming ability of freshly prepared chromatin was determined in a manner based largely on that described by Nakamoto *et al.* (1964). The following components (total volume 0.2 ml.) were mixed in tubes supported in an ice bath: 50 μmoles of tris-HCl buffer, pH 7.5; 1 μmole of MnCl₂; 10 μmoles of β-mercaptoethanol; 0.2 μmole each of ATP, CTP and GTP; 0.02 μmole of UTP and 0.2 μCi of [³H]UTP; 50 μg. of RNA polymerase. Finally, purified prostatic DNA (10 μg.) or

chromatin equivalent to 8–24 μg . of DNA was added, and the tubes were incubated at 30° for 10 min. The tubes were returned to the ice bath, and the reactions were terminated by the addition of 1.0 ml. of 5% (w/v) trichloroacetic acid. The samples were collected on Millipore filters as described above. Counts were corrected for background and for the incorporation of UTP in the absence of polymerase and expressed as μmoles of UTP incorporated/10 min./100 μg . of DNA. The incorporation with chromatin was then compared with that achieved with purified prostatic DNA, which possessed twice the priming ability of the best commercial samples of DNA. Before these priming studies were made it was established that the RNA polymerase was free of ribonuclease activity and that the amount of incorporation was strictly proportional to the amount of DNA added.

Examination of RNP particles and RNA on sucrose gradients. (a) RNP particles. The RNP suspensions were examined in a system essentially based on that of Abell, Rosini & Ramseur (1967). In the present work, however, the gradients were made up manually and scaled down to an overall volume of 4.75 ml. Sucrose solutions were made up in medium A. Each gradient initially consisted of six layers (0.75 ml. each) of 50, 35, 30, 25, 20 and 15% (w/v) sucrose. The gradients were equilibrated overnight in the cold so that the freshly prepared suspension of RNP particles could be applied immediately to a stable gradient. The gradients were centrifuged in the swinging-bucket rotor (cat. no. 9750) of a Griffin Christ ultracentrifuge at 18000 rev./min. (25000 g_{av}) for 4 hr. at 4°. The tubes were punctured with a 28 G hypodermic needle and fractions (3 drops, approx. 0.20 ml.) were collected and monitored by extinction analysis at 260 $m\mu$.

(b) ^3H -labelled RNA. Preparations of nuclei (500–700 μg . of DNA) were labelled with [^3H]UTP and extracted with phenol by the methods of McIndoe & Munro (1967) and Perry (1962). The labelled RNA was supplemented with 50–100 μg . of rat liver RNA and layered on to continuous 8–40% (w/v) sucrose gradients, prepared as described above. After centrifugation at 38000 rev./min. (110000 g_{av}) for 6 hr. at 4° the gradients were monitored as described above. The individual fractions were finally treated with an equal

volume of 2N-NaOH at 60° for 2 hr., dissolved in 15 ml. of phosphor and counted.

Fractionation of nuclear-associated proteins. Samples of nuclei, equivalent to 50–250 μg . of DNA, were concentrated by centrifugation at 700 g in pointed tubes of 1.0 ml. capacity in a microcentrifuge (Microchemical Specialties Co., Berkeley, Calif., U.S.A.). The nuclear pellets were re-suspended in 0.75 ml. of 5 mM-tris-HCl buffer, pH 7.5, at 0° and centrifuged 5 min. later at 4500 g . The clear supernatant, termed the neutral protein fraction, was aspirated via a constriction pipette and the residue was resuspended in 0.75 ml. of ice-cold 0.2N-HCl. Centrifugation 5 min. later yielded a soluble basic protein fraction and a residue that contained over 90% of the DNA originally present and the residual acidic protein fraction. The final residue was dissolved in 0.75 ml. of 1N-NaOH. Each fraction was analysed for protein and the final residue for DNA. The ratios of total protein and of individual protein fractions to DNA were calculated.

RESULTS

Purity of the nuclei. The recoveries of DNA in the nuclear preparations were 42–49%, 18–20% and 33–40% in liver, lung and prostate tissue respectively. The low DNA recovery from lung was almost certainly due to incomplete homogenization, since the main loss of DNA occurred during the filtration of the initial homogenates. Less than 5% of the total cytochrome oxidase and glucose 6-phosphatase activities was found in the final preparations of liver and lung nuclei, indicating high biochemical purity. Glucose 6-phosphatase could not be detected in prostatic homogenates, and glucose 6-phosphate dehydrogenase was used as a measure of extranuclear contamination. The prostatic nuclei did not contain any traces of activity of this enzyme or of cytochrome oxidase.

Effect of age on nuclear RNA synthesis. The synthesis of RNA was measured in purified nuclei isolated from liver, lung and prostate of mice of 6 and 30 months of age. The results are summarized in Table 1. RNA polymerase activity in lung nuclei was consistently low and variable, and no reliable indication of the effect of aging could be ascertained. In liver and prostate tissue, however, aging was accompanied by a significant decrease in RNA polymerase activity.

Changes in nuclear protein/DNA ratios with age. The proteins associated with purified liver, lung and prostatic nuclei were separated into neutral, basic and acidic fractions. The variations in the ratios of the total protein and of the individual protein fractions to DNA of tissues of various ages are shown in Figs. 1(a) and 1(b). The total protein/DNA ratio for liver nuclei did not alter appreciably with age, whereas the ratio for lung nuclei fell during senescence. Prostatic nuclei represented a difficult category in which the high protein/DNA ratio in the young animals was dramatically lost in old age.

Table 1. *Effect of age on RNA polymerase activity in purified nuclei*

Details of the assay are given in the Materials and Methods section. Results are given as the means $\pm$ s.d. of at least three separate determinations with pooled tissue from four to six animals per experiment. Enzyme activity was calculated as μmoles of UTP incorporated/15 min./100 μg . of DNA.

Tissue	RNA polymerase activity		P
	Young (6 months)	Old (30 months)	
Liver	43.8 $\pm$ 3.1	29.0 $\pm$ 1.2	< 0.01
Plus ribonuclease	0.7 $\pm$ 0.07	—	
Plus deoxyribonuclease	1.9 $\pm$ 0.1	—	
Lung	4.8 $\pm$ 1.4	3.9 $\pm$ 0.9	—
Prostate	14.2 $\pm$ 1.3	8.6 $\pm$ 0.6	< 0.01

Fig. 1. Changes in (a) the total nuclear protein/DNA ratio and (b) acidic protein/DNA and basic protein/DNA ratios in purified nuclei with age. $\square$ and $\blacksquare$, Liver; $\circ$ and $\bullet$, lung; $\triangle$ and $\blacktriangle$, prostate. In (b), $\square$, $\circ$ and $\triangle$ refer to basic proteins and $\blacksquare$, $\bullet$ and $\blacktriangle$ to acidic proteins.

Table 2. *Effect of age on the priming ability of chromatin*

For details of the assay system see the Materials and Methods section. Each result is the mean of three experiments in which chromatin was prepared from the pooled tissue from four animals. Counts in test samples were corrected for background and for the incorporation of UTP in the absence of RNA polymerase. Incorporation was calculated as disintegrations/min./100 μ g. of DNA, and is given as percentage incorporation/100 μ g. of purified prostatic DNA. This standard system incorporated 6.76 m μ moles of UTP/10 min./100 μ g. of purified DNA.

Tissue	Relative priming ability (prostatic DNA=100)		Ratio old/young
	Young (6 months)	Old (30 months)	
Liver*	2.9	2.7	0.93
Lung	7.3	5.3	0.73
Prostate	22.1	12.2	0.55

* In some experiments with liver chromatin, twice the usual amount of RNA polymerase was added.

When the ratios of the acidic fractions and of the basic fractions to DNA were examined (Fig. 1b) a general decrease in both ratios with age was established in all the nuclei studied. However, the decrease in the acidic protein/DNA ratio in the prostate was particularly pronounced, so much so that the decrease in the total protein/DNA ratio in these nuclei from old mice could be largely attributed to the selective loss of these proteins.

Priming ability of chromatin from young and old animals. The priming ability of chromatin isolated

from young and old mice was measured and compared with that of highly purified prostatic DNA (Table 2). Considerable differences were found in the priming ability of the chromatin isolated from different tissues of the same age and also between chromatin from young and old animals. Liver chromatin, even from young animals, was a poor primer and its efficiency was essentially unaffected by aging. Lung chromatin was a more efficient primer, but senescence was accompanied by a significant decrease in priming ability. The most efficient primer was prostatic chromatin, and again aging resulted in a pronounced decrease in priming ability. As noted in the Materials and Methods section, there was a marked difference in the physical appearance of the chromatin preparations. Lung and prostatic chromatin was filamentous and easily dispersed in water, yielding a viscous solution. In contrast, liver chromatin was amorphous and gave rise to a solution (of low viscosity) only after persistent agitation.

Attempts to establish which species of RNA was affected by aging. (a) Extraction and separation of labelled RNA. Purified prostatic nuclei, labelled with [^{3} H]UTP, were extracted with phenol in two different sets of experimental conditions. The resultant profiles of prostatic RNA were unsatisfactory owing to the instability of the extracts.

(b) Examination of the RNP particles in aging tissues. The sedimentation profiles of the RNP particles from tissues of young and aged mice are given in Fig. 2. The RNP particles from tissues of young mice were separated in many discrete peaks with a significant proportion of heavy material at the bottom of the gradients. In liver the bulk of the particles sedimented about half way down the gradient.

Fig. 2. Sedimentation profiles of RNP particles from tissues from young and old mice. Sedimentation was from right to left. Profiles in the left-hand column [(a), (c) and (e)] are from tissue from young animals and those in the right-hand column [(b), (d) and (f)] are from tissue from old animals. (a) and (b) Liver; (c) and (d) lung; (e) and (f) prostate.

Senescence of all the tissues studied was accompanied by a considerable change in the RNP profiles. There was a pronounced shift in the sedimentation of the RNP particles towards the upper regions of the gradients, suggesting that the particles possessed lower sedimentation coefficients. The change in sedimentation behaviour was particularly apparent with RNP from the prostate of old mice (Fig. 2f).

The proportion of RNA in the post-microsomal fraction of tissues was also affected by aging (Table 3). In the tissues from young animals studied, this fraction contained 8–11% of the total RNA present. With age, however, the contribution of this fraction rose to 35–40%. Samples of 105 000g supernatants were further treated by either dialysis or gel filtration before RNA analysis. Much of the RNA was lost from prostatic-cell supernatant fractions during prolonged dialysis, but in the brief gel-filtration

procedure the recovery of RNA was essentially complete. These results further illustrated the instability of prostatic RNA during conventional procedures of isolation. When examined by electrophoresis in 10% polyacrylamide gels, the RNA in the 105 000g supernatant migrated at the same rate as yeast RNA. The recoveries of RNP particles from the sedimentable RNA fraction were satisfactory, but losses were apparent in prostatic homogenates.

DISCUSSION

Despite the biochemical purity of the various nuclear preparations, as judged by enzyme tests, the recovery of DNA was not particularly high. Widnell & Tata (1964), for example, reported a DNA recovery of 62% by their method for purifying liver nuclei. Further, the recovery of nuclei might

Table 3. *Percentage of RNA present in microsomal and post-microsomal fractions of aged tissue homogenates*

Ribonuclease activity in tissue homogenates									
RNA content (mg./g. of tissue)		Recovery in microsomal fraction		Recovery in post-microsomal fraction			Yeast RNA hydrolysed (μg./10 min./100 μg. of homogenate protein at 37°)	Ratio old/young	
		In original pellet	As RNP particles	In original supernatant	After gel filtration	After dialysis			
(a) Young tissues (6 months)									
Liver	11.33	90.0	82.7	10.0	—	—	12	1.56	
Lung	8.39	89.8	74.8	10.8	—	—	40	1.12	
Prostate	2.09	91.4	43.3	8.6	—	—	78	1.58	
(b) Old tissues (30 months)									
Liver	7.88	64.5	55.2	35.5	34.0	21.9	19		
Lung	7.72	63.1	52.2	36.9	34.2	32.8	44		
Prostate	1.73	60.0	16.1	40.0	32.0	7.5	122		

Tissues were homogenized as described in the Materials and Methods section and separated into 105 000g pellet and supernatant fractions. The recovery of sedimentable RNA as RNP particles was calculated from the sucrose gradients. The RNA content of the 105 000g supernatant fraction was also measured after gel filtration or prolonged dialysis. All results are quoted as percentages of the RNA present in the original homogenates. The conditions of the ribonuclease assay are given in the Materials and Methods section; the results are the means of three determinations with pooled tissue from four animals per experiment. All these results were within $\pm 7\%$.

vary with age, but this point has not been rigorously pursued.

Aging of tissues results in a decreased ability to synthesize RNA (based on the assay of RNA polymerase in purified nuclei). Comparable data are few. Conflicting results were presented by Detwiler & Draper (1962) and Samis, Wulff & Falzone (1964) on the incorporation of radioactive precursors into liver nuclei from old animals; their approaches were very indirect and based on the specific radioactivities of extracted RNA. By a similar approach to that used in the present work, Devi, Lindsay, Raina & Sarkar (1966) reported a decrease in the synthesis of RNA in liver nuclei from old animals. The decrease in RNA synthesis could be attributed to a loss of RNA polymerase, deleterious changes in the DNA template or the loss of other unknown regulatory factors with age. Devi *et al.* (1966) suggested that their results could be satisfactorily explained, not by an age-associated loss of RNA polymerase, but rather by changes in the DNA template.

Huang & Bonner (1962) showed that histones could inhibit DNA transcription, and this inhibition can be counteracted by acidic nuclear proteins (Langan, 1967). The changes in the nuclear-associated proteins with age were in fair agreement with these observations, since the overall loss of acidic nuclear proteins in all the nuclei coincided with a decrease in RNA synthesis.

Aging resulted in a marked fall in priming ability in lung and prostatic chromatin but not in liver chromatin. Despite the difficulties encountered in dissolving liver chromatin (see the Results section), its low priming ability could not be attributed to precipitation during incubation (Bonner & Huang, 1966a). The proportion of DNA transcribed in prostatic and liver chromatin from young animals agrees with the findings of Liao & Lin (1967) and Thaler & Villee (1967) respectively. Hahn (1964) found that the DNA from the thymus of aged oxen had an increased stability to thermal denaturation, and attributed this to a firmer binding of histone to DNA. He further postulated that these changes in histone-DNA binding could lead to a decrease in DNA transcription. The citric acid-washing procedure should strip the lysine-rich histone from DNA (Bonner & Huang, 1966b) unless it is particularly firmly bound, thus exposing more potential sites for RNA synthesis. It therefore seems possible that aging affects the physicochemical state of the deoxyribonucleoprotein complexes in lung and prostate but not in liver.

No evaluation of the *S* values of the peaks in the RNP profiles was attempted, since only a knowledge of the general distribution was needed. The profile of the RNP particles from the livers of young mice was similar to the distribution of liver polysomes reported by Wettstein *et al.* (1963) and Webb,

Blobel & Potter (1964), but differed significantly from the results of Murthy (1966) and Abell *et al.* (1967), who found that the bulk of liver RNP particles remained at the top of the gradients. A comparison of results from different Laboratories is often difficult, and in this case the disparities may be due to different methods of preparation and such factors as the temperatures at which the various procedures were conducted (Wettstein *et al.* 1963). The method used in the present work was specifically developed for prostatic RNP particles (Liao & Williams-Ashman, 1962), and was applied to the other tissues for complete standardization of experimental procedure. The profile of prostatic RNP particles is similar to that reported by Mangan, Neal & Williams (1967), for there was an apparent parity between the amount of the two slower-moving peaks, which they established had $S_{20,w}$ values of 79s (monomer) and 125s (dimer) respectively. Most of the RNP particles from all the aged tissues were found at the top of the gradients, which could perhaps be explained by a depletion of messenger RNA with age (Mansbridge & Korner, 1966). One inherent problem, however, encountered in the isolation of RNP particles is the ease with which the messenger RNA can be hydrolysed enzymically, resulting in an increase in the number of ribosome monomers (Munro, Jackson & Korner, 1964). Such degradation could explain the present results, as an increase in total ribonuclease activity was found in homogenates of tissues from aged animals, particularly liver and prostate gland. The degradation may be further potentiated by the release of enzymes from the more fragile lysosomes of cells from aged animals during homogenization (de Duve, 1963). Other evidence, however, suggests that the RNP profiles are not necessarily preparative artifacts, for electron micrographs of prostate glands from old animals indicate a breakdown of endoplasmic reticulum and disorganization of the ribosomes (C. Rowlatt, personal communication). The losses of RNA in the post-microsomal supernatants during prolonged dialysis were probably related to the increased amounts of ribonuclease in the tissues from old animals. On the other hand, the essentially complete recovery of the RNA in the brief gel-filtration procedure indicated that the material was initially of high molecular weight. These results indicate that cells from old animals possess an abnormally high proportion of non-sedimentable RNA, which on electrophoretic examination contained transfer RNA.

It is tempting to suggest that aging is accompanied by a decrease in ribosomal RNA synthesis, for, apart from the increase in non-sedimentable RNA, other workers have shown that in the assay conditions of RNA polymerase at low ionic strength, as employed here, the product is mainly ribosomal

RNA (Widnell & Tata, 1966; Liao, Barton & Lin, 1966). Confirmation of this concept would demand the use of nucleoside 5'-[α - ^{32} P]triphosphate and base-ratio analysis.

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