Influence of age and cataract formation on the ribonucleic acid of the lens

II. Changes in concentration and distribution of RNA in rabbit lenses during the first year of life

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The concentration of RNA in rabbit lenses decreases continuously during the first year of life. This is due mainly to the decrease in the microsomal RNA which sediments at and above 60,000 G, and parallels the decrease in the rate of synthesis of lens proteins. The rabbit lenses contain an RNA fraction which is linked to the albuminoid. In 2-week-old rabbits this protein fraction contains 1 to 3 per cent RNA. The concentration of RNA in the albuminoid decreases sharply during the first year of life. Soluble RNA represents 30 to 40 per cent of the total RNA in 2-week-old and more than 75 per cent in 1-year-old animals. The $HClO_4$ extracts of soluble lens proteins remaining after removal of all cytoplasmic particles and the so-called acid-soluble nucleotides contain nucleotides which, in the orcinol reaction, react like purine mononucleotides substituted in position 5.

It has been previously reported[1] that lenses of rats contain ribonucleic acid (RNA) which amounts to about 300 μg in 100 mg. lens in 5-week-old animals. The concentration of RNA continuously decreases with age and at the end of the life-span drops to about one fourth to one third of the value found in 5-week-old rats. Furthermore, it has been shown that the RNA of the lens is heterogeneous and distributed among cytoplasmic particles of varying sedimentation constants. In addition, a fraction corresponding to the soluble RNA (SRNA) in other tissues was found in the lens. One fraction among the particulate RNA was found to be associated with the albuminoid, and, therefore, in particularly large amounts in the nucleus of the lens. It appears questionable whether this fraction has a correlate in other tissues, such as the liver and kidney since in the latter it cannot be distinguished from the nuclear RNA. Whereas the amount of this last fraction increased with age, the other particulate RNA fractions were shown to be sharply depleted in the rat after the first year of life, and to disappear almost completely from the microsomal particles at the end of the life-span.

It seemed of particular interest to investigate whether the same pattern of distribution of RNA and changes with age can be found in lenses of other species and at the same time to attempt a more detailed characterization of different RNA fractions, particularly of SRNA of the lens. For this reason the RNA in lenses of rabbits varying in age from 6 days to 12 months has

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been investigated and the present report deals with the results of these experiments.

Experimental

Animal material. The investigation was carried out on New Zealand albino rabbits. The very young animals were obtained before weaning together with their lactating, about 1-year-old mothers. Their ages were carefully controlled and varied between 6 and 17 days. Every group of young animals consisted of 5 to 6 animals of the same litter, or 12 animals from two litters. In addition, two sets of young adults—4 and 4½ months old —a series of 9-month-old rabbits, and a group of 1-year-old nonlactating animals were investigated. The ages of the mothers of the baby rabbits could not be ascertained, so the analytical data in their cases are correlated with the weight of the body and of the lenses. The animals were killed by ether. The globes were removed immediately after death and the lenses extracted under cooling with ice.

Analytical procedures. The lenses were homogenized by crushing with a glass rod in a five- or tenfold volume of distilled water precooled to 0° C. and the homogenate was immediately centrifuged at 3,000 r.p.m. (600 × G) for 30 minutes in the Spinco Preparative Ultracentrifuge at 0° C. The precipitate sedimenting at this speed contained the debris of the lens capsule and was assumed by analogy with the rat lens to contain the total albuminoid of the rabbit lens. One part of the supernatant homogenate was immediately removed and precipitated in ice with 5 per cent trichloroacetic (TCA) or perchloric acid ($HClO_4$). In the latter case, the homogenate was diluted with two parts of distilled water, and one part of 20 per cent $HClO_4$ was added. After standing for half an hour at 0° C., the precipitate was separated by centrifugation, the supernatant decanted, and the protein precipitate washed twice, first with 5 per cent and finally with 10 per cent perchloric acid at 0° C. The remaining supernatant was first centrifuged for 1 hour at 12,000 r.p.m. (10,000 × G) in the Spinco Preparative Ultracentrifuge, the precipitate separated from the supernatant fluid by decantation, and the latter again centrifuged first for 3 hours at 30,000 r.p.m. The precipitate was again removed, and the supernatant centrifuged again for 2 hours at 40,000 r.p.m. (105,000 × G). The supernatant solution remaining after the last centrifugation was again treated exactly like the initial homogenate, the precipitates (designated as Fractions 3,000, 12,000, 30,000, and 40,000) were washed once with water and then twice with 10 per cent perchloric acid to remove the acid-soluble nucleotides. RNA in all these protein precipitates was then extracted, according to the procedure of Ogur and Rosen,[2] by suspending the precipitates in 1N HClO precooled to 0° C. and leaving the suspension at 0° C. under repeated shaking for varying periods of time. It has been found that the extraction of RNA of rabbit lenses is much slower under these conditions than was observed with other tissues, such as liver and kidney. After a 24 hour incubation period only about 50 to 60 per cent of RNA appeared to be extracted, and only after an extraction of 90 hours between 88 and 96 per cent of RNA could be recovered. In general, therefore, the extraction was continued for 4 days and the values for RNA obtained in the supernatant solutions were assumed to represent 90 per cent of the total RNA. In some experiments, determinations of RNA were carried out separately on fractions extracted after 24 hours and 4 days.

RNA was determined in the $HClO_4$ extracts in two different ways, first by the orcinol reaction, according to Dische and Schwartz.[3] Dichromatic readings were carried out at 670 mμ and 580 mμ against two kinds of standards, namely, a commercial preparation* of yeast RNA, and a preparation of soluble yeast RNA† prepared according to the procedure of Zamecnik and co-workers.[4] Both preparations were assayed by measuring their E_{260} in 1N $HClO_4$ and calculating the content in RNA from the theoretical E_{260} for RNA (10,800) As a rule, with the orcinol reaction, spectrophometric readings were carried out at two different time intervals; first after heating for 3 minutes, reading, and then heating again for an additional 17½ minutes and repeating the readings at the two wave lengths. In this way it was possible to measure the rate of the color development of the RNA fractions of the lens and compare it with the standard of RNA. This rate was found to be identical for the commercial yeast RNA and soluble RNA as well as for the preparation of SRNA from hog liver.‡ Furthermore, the readings at the two wave lengths permitted comparison of the ratios of the extinction coefficients for various RNA fractions at two wave lengths with those of corresponding standards, and testing in this way how far the absorption curves in the orcinol reaction of our preparations agree with those of RNA standards. In addition, the RNA in all fractions was determined by ultraviolet absorption which was measured in the whole range between 240 and 290 mμ. These measurements were possible only when no TCA had been used for precipitation of the homogenate or washing of pre-

*Supplied by Nutritional Biochemicals Corporation, Cleveland, Ohio.

†Supplied by Dr. R. W. Chambers, Department of Biochemistry, New York University.

‡Supplied by Dr. R. W. Chambers.

cipitates because it appeared that as is well known, impurities in TCA which show a strong absorption in the ultraviolet were found to form a firm combination with proteins and could not be washed out with perchloric acid except after a very prolonged incubation period. N determinations were made by the micro-Kjeldahl method.

Results

Changes with age in the concentration of total RNA and of its content in individual lenses. In the first series of experiments the total concentration of RNA was determined in 20 groups of animals ranging in age from 6 days to about 1 year. The total concentration of RNA in the lens was calculated as the sum of RNA separately determined in the homogenate after removal of the fraction sedimenting in 30 minutes at 3,000 r.p.m. and in the 3,000 fraction. The results of these determinations are listed in Table I. The table shows that there is a continuous decrease in the concentration of the total RNA after 12 days from an average of about 160 mg. per cent of wet weight to about 40 mg. per cent in the 1-year-old animals. The content of the individual lenses, however, increases continuously from about 130 μg to about 250 μg in animals of the same age.

Distribution of RNA between different parts of the lens and different cytoplasmic fractions. In another series of experiments RNA was determined in the four main cytoplasmic particulate fractions which sediment at 600, 10,000, 65,000, and 105,000 × G, and a fraction in the supernatant, which can be considered as corresponding to the so-called SRNA isolated from other tissues. The determinations of RNA in fractions of rabbit lenses, however, proved to be more difficult than in those from rat lenses because of a considerable instability of the lenticular RNA in the rabbit as compared with that in the rat. This instability proved to be due to an overlapping of continuous synthesis and breakdown of RNA under the conditions of fractionation. The breakdown processes increased considerably not only with higher temperature but also with higher dilution of the homogenate. By homogenizing the lens in no more than fivefold its amount of water and keeping the temperature during the homogenization and fractionation near 0° C. it has

Table I. Total RNA content of lenses of rabbits at various ages

Experiment No.	*No. of lenses*	*Net weight of lens (mg.)*	*Age*	*RNA (µg/lens)*	*RNA (µg/100 mg. lens)*
I	12	51.0	6 days	63*	132.5*
II	10	70.5	12 days	121.0	172.0
III	12	75.0	12 days	112.5	149.5
IV	12	71.5	12 days	98.0	137.0
V	12	75.0	14 days	150.0	149.0
VI	24	87.5	15 days	135.0	154.0
VII	5	291.0	17 days	185.5	203.5
VIII	6	307.0	3 months	125.0	76.5
IX	8	338.0	4 months	294.0	87.0
X	5	459.0	9 months	202.0	44.0
XI	8	450.0	9 months	207.0	45.0
XII	8	477.0	9 months	232.0	48.4
XIII	8	454.0	9 months	272.0	59.5
XIV	2	499.0	9 months	245.0	48.2
XV	2	476.0	9 months	295.0	61.9
XVI	4	595.0	About 1 year	193.0	34.2
XVII	4	625.0	About 1 year	293.0	46.9
XVIII		636.0	About 1 year	303.0	47.4
XIX		640.0	About 1 year	285.0	44.6
XX		645.0	About 1 year	186.0	28.8

*Calculated as a sum of RNA in fractions.

been possible to reach a constant level of RNA during the whole fractionation procedure in the cortical region of the lens in 1-year-old animals and in the nuclear part of the 2-week-old animals. The stability of RNA due to the mutual compensation of synthesis and breakdown also showed considerable variation from one animal to another so that it was possible in some animals to recover all the RNA present before fractionation as a sum of that found in individual fractions, even when a tenfold amount of water was used for homogenization and the fractionation carried out on the whole lens. It must be noted, however, that the breakdown of RNA during the fractionation almost exclusively affected the microsomal fraction coming down at and above 30,000 r.p.m. and the SRNA. On the other hand, it could be shown that the fraction which comes down at 600 G's, and can be considered as representing the albuminoid of the lens in the nuclear part of older rabbits, apparently consists of two subfractions: one which corresponds to about one half to two thirds of the total fraction sedimenting at 600 G's comes after centrifugation of 30 minutes; the rest sediments at a much slower rate, and the complete sedimentation requires up to 4 hours of centrifugation. During this prolonged centrifugation a further breakdown of RNA was to be expected. To avoid this difficulty the second part of the 3,000 fraction was included in the 12,000 fraction and spun down with the latter. The fractionation procedure was applied not only to the whole lens, but also to four different parts of the lens, namely, the whole cortical region which, by weight, corresponded to about 60 per cent of the total weight of the lens; the nuclear part which corresponds to about 40 per cent of the lens and, in one case, to the equatorial part which corresponded to about 45 per cent of the total weight of the lens; and the remaining polar part. The results of the determination of RNA in various parts of the lens and in different cytoplasmic fractions, are listed in Table II. In these experiments the total RNA determined in the homogenate immediately after removal of the 3,000 fraction was compared with the RNA recovered from different parts of the lens and different fractions. In no case did the maximum loss during this fractionation procedure exceed one third of the initial value, and this loss was due to the breakdown of RNA in the nuclear part of the older animals and in the peripheral part of the 14-day-old animals. The total amount of the 3,000 fraction in the lens increases from a value between 8.1 and 22.2 μg in about 2-week-old animals to a value varying between 22.4 and 47.2 μg in 1-year-old animals. Additional experiments on 5 groups of animals about 2 weeks of age and on 2 more groups of two 1-year-old animals each, in which, because of far-reaching breakdown of RNA during fractionation, only the 3,000 and 12,000 fractions were determined, showed that the average amount of albuminoid RNA rose from 15 μg per lens in 8 experiments to 23 μg per lens in 5 experiments on 1-year-old animals, whereas in 5 experiments on 9-month-old animals it was 18 μg per lens. Still more pronounced changes appear in 3,000 RNA values calculated in per cent of protein. Whereas in 2-week-old animals the percentage of RNA in this fraction varied between 1 and 3 per cent, it varied in the 1-year-old between 0.1 and 0.34 per cent, and in the 9-month-old animals intermediate values were again found. The 12,000 fraction of RNA shows much greater variations than the 3,000 fraction; in general, it appears to reach a maximum in 9 months.

The microsomal fractions which sediment at 30,000 and 40,000 r.p.m. also show a very wide scattering with no significant trend so far as the total amount per lens at different ages is concerned. If, however, instead of the total amount per individual lens the amount per 100 mg. lens is considered, there is a very significant decrease with age. The values for 2-week-old animals range from 20 to 80 mg. per cent, drop in the 1-year-old animals to 2 to 3 mg. per

Table II. Distribuiton of RNA among various parts and cytoplasmic fractions of rabbit lenses at various ages

Experiment No.	Age	Wet weight of lens (mg.)	Fractions									Total RNA (per 100 mg. per lens)	
			3,000		12,000		30,000		40,000		SRNA		
			RNA (% of protein)	RNA (μg/ lens)	RNA (% of protein)	RNA (μg/ lens)	RNA (% of protein)	RNA (μg/ lens)	RNA (% of protein)	RNA (μg/ lens)	RNA (μg/ lens)	Original value	Recovered value
I	12 day	75.0	1.05	8.1	17.2	13.8	26.5	15.1			45.0	149.5	110.0
II	14 day	75.0	3.15	22.2	33.0	9.9	23.8	52.2	20.3	12.5	43.3	199.0	176.0
		a. Periphery 45	1.85	5.4	35.0	8.2	29.0	27.2	18.6	6.95	19.8	188.2	150.4
		b. Nucleus 30	5.95	16.8	29.0	1.7	16.1	25.0	22.7	5.5	23.5	215.3	214.7
III	17 day	91.0	1.0	16.6	19.2	12.8	54.0	42.5	15.6	14.2	44.0	202.4	149.6
IV	9 months	454.0	0.27	12.7	1.25	50.9	2.7	68.0	4.7	11.7	175.0	59.8	67.0
V	9 months	477.0	0.18	21.0	0.45	42.5	0.33	18.3			120.0	48.4	42.5
VI	9 months	499.0	0.22	26.3	0.18	26.8	0.29	10.4			80.0	48.2	32.9
		a. Equatorial 334	0.29	18.4	0.38	2.7	1.95	4.95			37.9	47.5	
		b. Polar 165	0.14	7.9	0.16	24.1	0.16	6.0			42.1	49.5	
VII	9 months	476.0		14.2	1.5	47.6	0.3	18.3			107.0	61.8	39.4
		a. Periphery 206		7.2	7.2	4.9	3.9	7.0			36.6	43.9	
		b. Nucleus 270		7.0	1.4	42.7	0.2	11.3			70.4	75.2	
VIII	1 year	625.0	0.34	47.2	0.25	18.8	0.22	38.0			163.0	46.9	40.7
		a. Periphery 410	0.40	34.7	1.22	5.3	1.2	24.5			124.0		
		b. Nucleus 215	0.14	12.5	0.2	13.5	0.14	13.5			39.0		
IX	1 year	595.0	0.1	28.3	0.19	30.8	1.15	16.3	0.55	9.7	121.0	34.2	38.5
		a. Periphery 360	0.1	6.5	0.85	7.7	4.1	7.2	4.5	7.9	106.0	31.2	39.4
		b. Nucleus 235	0.1	17.3	0.55	23.1	0.06	9.1	0.1	1.8	15.0	38.3	23.7
X	1 year	671.0	0.11	22.4	0.18	30.2	0.1	16.7			252.0		47.8

cent, and are between 2.5 and 14 mg. per cent at 9 months of age. There is also a marked decrease between the 2-week-old animal and the older animal in the percentual content of RNA in the proteins of these fractions; the very young animals show very high values, between 16 and 54 per cent, against 0.1 to 1.15 per cent in 1-year-old animals and 0.15 and 2.7 mg. per cent at 9 months of age. SRNA increases from 21 to 45 μg per lens to 200 to 250 μg per lens in 9- and 12-month-old animals. The two latter age classes do not differ significantly in this respect, but the concentration of SRNA in the lens declines only slightly between 14 days and 1 year from about 40 to about 30 mg. per cent.

The total concentration of RNA in the nucleus is somewhat higher than in the peripheral part of the lens in all three age classes, and this was found to be true in the one experiment in which the polar and the equatorial parts of the lenses of 9-month-old animals were compared (Table II). This was not the case in the lens nucleus of one 1-year-old animal in which there was a very significant breakdown of RNA during the fractionation. As far as the RNA of various cytoplasmic fractions is concerned, no significant differences could be observed between the total amounts in the nucleus and periphery of individual lenses. The values calculated in per cent of proteins, however, are very significantly smaller in the nucleus than in the periphery in older animals. In one experiment on the 14-day-old rabbits the situation appears reversed relative to the 3,000 fraction. Here the RNA in per cent of protein is higher in the nucleus. In this instance it does not differ significantly from the protein content in other fractions of the peripheral part.

Comparison of the composition of fractions of lens SRNA with yeast RNA. In the orcinol reaction of pentoses only the ribose of the purine nucleotides reacts significantly. As the hydrolysis of the RNA by acid produces ribose nucleotides substituted in position 3, the rate of the color development in this reaction with RNA is identical with that observed with

Table III. Values for RNA content (μg/100 mg. lens) obtained in various cytoplasmic fractions by the orcinol reaction (OR) and by ultraviolet absorption at 260 mμ (UV) and for ratios E_{670} to E_{580} in the 3 and 20 minutes orcinol reaction in per cent of ratios for the standard

			Fractions					
			12,000		*3*	*SRNA*		*3*
Experiment No.	*Age*	*Net weight (mg./lens)*	*OR*	*UV*	*20*	*OR*	*UV*	*20*
I	6 days	50.5	18.0	19.6	108	48.4	44.5	120
II	12 days	75	18.4	21.5	104	60.5	55.9	128
III	14 days		4.85	5.4		44.0	44.9	
a. Nucleus								
b. Periphery			10.0	10.4		29.3	37.6	
IV	14 days	80.5	13.5	15.0	108	39.8	35.5	123
V	15 days	88	12.0	62.0		22.5	25.6	113
VI						48.8	53.6	
VII	24 days	117	17.0	16.2		55.4	55.4	111
VIII	4 months	338	11.6	12.0		33.7	36.1	
IX	9 months	477	15.6	15.6	102	28.1	25.3	130
a. Nucleus								
b. Periphery						17.8	16.35	123
X		556				11.7	8.6	
XI	1 year	636	8.2	8.6		36.4	19.8	145
XII	1 year	625			106	32.7	31.1	133
XIII	1 year	595				31.0	25.2	
Periphery								

adenosine-3-phosphate, and differs significantly from that observed with adenosine-5-phosphate. In the determination based on the ultraviolet absorption, on the other hand, purine as well as pyrimidine nucleotides contribute to the absorption. E_{260} of the latter nucleotides is only about one half of that of the first ones. The ratio of E_{260} to the extinction coefficient in the orcinol reaction will, therefore, be different for purine nucleotides and RNA. Fifty to sixty per cent of the pentose nucleotides extracted by 10 per cent $HClO_4$ at 0° C. from lens homogenates are nondialyzable and the above-mentioned ratio for them is identical, or nearly so, with that of yeast RNA (Table III). These extracts, therefore, can be assumed to contain polynucleotides consisting of purine and pyrimidine nucleotides in a proportion similar to that in RNA. The ultraviolet absorption of guanylic and cytidilic acids differs from that of adenylic and uridylic acids. The first two have a significantly higher extinction coefficient in acid solution of 280 mμ. The form of absorption curves of RNA preparations will, therefore, vary with the ratio of the sum of guanylic and cytidilic acids to that of adenylic and uridylic acids. In Fig. 1 the absorption curves of 3,000, 12,000, 30,000, and SRNA fractions from the rabbit lens are compared with those of RNA and SRNA from yeast. The absorption curves of the two yeast RNA preparations are not identical. The absorption curve of the 12,000 fraction from lens has a slope almost identical with that of yeast RNA (between 260 and 290 mμ), the absorption curve of SRNA and the 3,000 fraction from the lens nucleus are almost identical with SRNA from yeast. The cortical SRNA from lens and the 30,000 fractions show significant deviations from yeast RNA in their absorption curves. More significant differences between SRNA from rabbit lenses and the RNA from yeast and other cytoplasmic fractions from lenses appear when they are compared relative to the rate of the color development in the orcinol reaction. The results of the experiments are listed in Table III in which the ratio of ϵ_{670} to ϵ_{580} in the orcinol reaction after heating for 3 minutes and 20 minutes, respectively, were determined for SRNA, and the 12,000 fraction from the lenses of rabbits of various ages with the same ratio of yeast RNA which was found to be identical with that of yeast SRNA. In the 12,000 fraction, this ratio differs from that of yeast RNA by no more than 2 to 8 per cent, which is not significant. In SRNA, on the other hand, this ratio is 11 to 45 per cent higher without exception, the average increase from 9 experiments being 27 per cent.

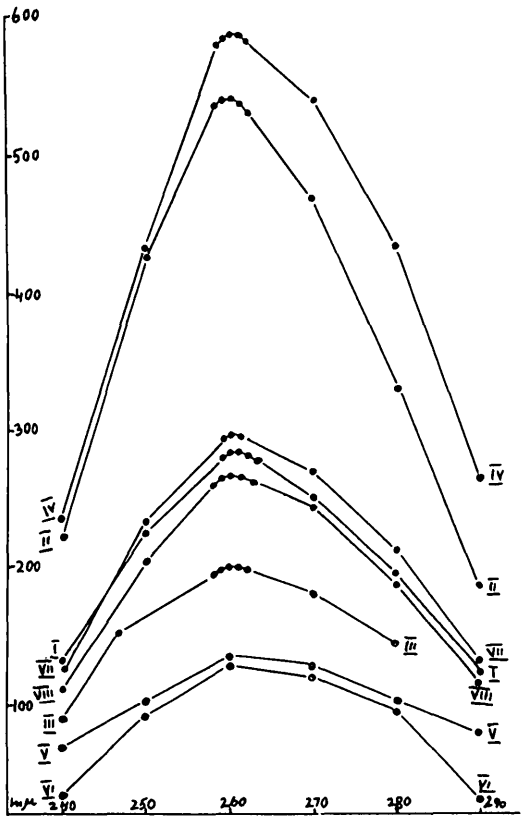

Fig. 1. Absorption curves of RNA from various cytoplasmic fractions and of standards in $HClO_4$. *I,* Nuclear SRNA. *II,* Standard of yeast RNA. *III,* Cortical SRNA. *IV,* Nuclear RNA from 12,000 fraction. *V,* Nuclear RNA from 30,000 fraction. *VI,* Cortical RNA from 30,000 fraction. *VII,* Nuclear RNA from 3,000 fraction. *VIII,* Standard of yeast SRNA.

Discussion

In any attempt to compare the content and distribution of RNA in lenses of rats and rabbits, it is first of all necessary to compare animals at ages which can be regarded as more or less biologically rather than chronologically equivalent. One way is to compare animals at ages which are identical fractions of the total life-span. For the rat, the latter can be assumed to be 3 years. The life-span of the rabbit does not appear to have been determined with the same accuracy. But on the basis of data in the literature we can assume, for our purposes, 7 years as the maximum life-span of this animal. Thus, each time interval in the life of the rat would correspond to a 2.3 times as long interval in the life of the rabbit. The lenses of 9-month-old rabbits would appear comparable with those of about 4-month-old rats.

Our results show that the over-all concentration of RNA in the rabbit lens decreases continuously from the moment of birth during the whole time interval of the first year of the animal's life. The total amount of RNA in the lens of the rabbit on the other hand increases continually during the same time interval. This increase appears to level off between the ages of 4 and 9 months. The conditions in the rabbit lens relative to the concentration and the amount in the individual lens are very similar to conditions found in the rat during the first 4 to 5 months of life. From the beginning the concentration of RNA in the rabbit lens is significantly smaller than in the lens of the rat and its rate of decrease is much steeper. The total amount of RNA in the lens, on the other hand, is significantly higher in the rabbit, which is due to the fact that the size of the rabbit lens at corresponding ages is about 10 times larger. The percentual increase in the amount of proteins in the individual lens is far greater in the rabbit than in the rat. There appears, therefore, to be no correlation between either the relative concentration of RNA or its amount in the individual lens and the relative rates of protein synthesis in the two species.

Further significant differences between rabbit and rat lenses appear when the distribution of RNA among various intracellular particles and the cytoplasm itself is considered. In the rat at all ages 30 to 60 per cent of the total RNA appears associated with the albuminoid (3,000 fraction). In the rabbit, on the other hand, the albuminoid-linked RNA presents no more than 4 to 10 per cent of the total RNA of the lens. This is partly due to a lower concentration of albuminoid in rabbit lenses, and in older animals partly to a lower content in RNA of the albuminoid. The lower level of RNA in the albuminoid in rabbit lenses is paralleled by a much lower relative rate of its formation after the fourth month of life. It appears possible, therefore, that the lower concentration of RNA in the albuminoid fraction of the rabbit and the lower relative rate of synthesis of albuminoid in the lens are related to each other. The lower over-all concentration of RNA in the rabbit lens compared with that in the rat lens is due exclusively to the lower concentrations of the particulate RNA. The concentration of SRNA is about the same in the lens of the 4-month-old rabbit as in that of the rat of comparable age.

The protein content of the individual rabbit lens increases about three- to four-fold between 14 days and 4 months. On the other hand, between 9 and 12 months of life the increase is only about 50 per cent. The rate of the protein synthesis during the first time interval is, therefore, about 6 to 8 times as high as that during the second. The over-all concentration of RNA in the lens at the same time goes down from about 160 mg. per cent (average of 7 groups of animals) to about 40 mg. per cent (average of 5 groups of animals). This indicates a certain parallelism between the over-all concentration of RNA and the rate of protein synthesis. This change in the concentration of RNA is not accompanied by the corresponding change in the concentration of SRNA, which de-

clines between 14 days and 1 year of age by no more than 25 per cent. The concentration of the albuminoid-linked RNA calculated per 100 mg. lens decreases significantly, but this fraction represents only a few per cent of the total RNA. The difference in the over-all concentration is mainly brought about by a very marked decline in the concentration of the microsomal fractions, as has also been shown to be the case during the aging of the rat lens. The decrease of RNA in this fraction is also very marked when RNA is calculated in per cent of the protein of the fraction. In young animals the content of the protein in the 30,000 and 40,000 fractions ranges between 18 and 50 per cent, whereas in 9-month- and 1-year-old animals, the values varied between 0.16 and 4.5 per cent. The very great scattering of the percentual content of RNA in the 30,000 and 40,000 fractions is due mainly to very great variations of these fractions in their protein content. This variability may be the result of a great instability in the soluble lens proteins of the lens nucleus of the rabbit. This phenomenon is now under investigation. The parallelism between the decrease in the microsomal RNA and the rate of protein synthesis agrees with the role which this RNA fraction is now generally assumed to play in the synthesis of cytoplasmic proteins.

The fact that the ratio of extinction coefficients in the orcinol reaction after 3 and 20 minutes' heating is significantly larger for SRNA than in other forms of RNA from rabbit lenses and the RNA from yeast indicates the presence of mononucleotides substituted in position 5 in these preparations of lenticular SRNA. As more than half of the SRNA proved to be nondialyzable, it is not possible to explain its behavior in the orcinol reaction as due to the presence of purine nucleotides as end groups of SRNA chains. Purine nucleotides substituted in position 5 would have to have their ribose linked by tertiary linkages to the phosphate in RNA chains. Another more probable possibility appears to be that ATP of the lens or its dephosphorylation products become attached to the basic groups of the lenticular proteins by linkages as firm as those of RNA itself. This last assumption appears necessary because when SRNA is extracted in a two-step procedure—after 24 and 96 hours the ratio of extinction coefficients after 3 and 20 minutes' heating is identical in the two extracts. The presence of such nucleotides may be at least partly responsible for the lower values for SRNA in older animals obtained by ultraviolet absorption as compared with those calculated from the orcinol reaction.

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Discussion

Dr. Jin H. Kinoshita, Boston, Mass. Dr. Dische in his usual thorough and analytical manner has opened a heretofore unexplored area in the biochemistry of the lens. I will confine this discussion to the significance of the presence of RNA within the lens. It was surprising to learn that RNA was distributed throughout the lens. That it is found in higher concentration in the lens nucleus than in the cortex was, to my mind, totally unexpected. The nucleus, consisting of old fibers, has been thought to be metabolically inactive. Yet the presence of RNA suggests that protein synthesis is taking place. How can we account for the presence of RNA in these lens fibers? It is gener-

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ally accepted that RNA is synthesized in the nucleus of the cell, where the DNA molecule serves as the master die or template for RNA synthesis. The RNA nucleotides are thus molded together and provide a set of working dies for the assembling of amino acids in a proper sequence to form proteins. It is quite obvious that the major portion of the lens has lost its nucleus and other cellular characteristics. This suggests that RNA must have originated either in the epithelial cells or in young cortical fibers and persisted through the aging process of lens fibers. More specifically, the possibility must be considered that, once RNA is formed, it is not broken down or resynthesized. That is, in most of the lens fibers, the RNA does not turn over. Let us extend these thoughts a step further. In those lens fibers which do not possess nuclei and in which no mechanism for resynthesis is available, what happens when the existing RNA is damaged or destroyed? Is it possible that this may lead to derangement of proteins which contributes to the process of opacification? I am sure we will profit from Dr. Dische's thoughts on these considerations.

Dr. Dische (closing). Dr. Kinoshita, in his remarks, pointed to a relevant question concerning the distribution of RNA in the lens. This question is how far the presence of large amounts of RNA in the center part of the lens can be regarded as biologically purposeful and necessary for the maintenance of the physicochemical state of lens fibers. This problem arises from the assumption that the old centrally located fibers of the lens which are devoid of nuclei do not show a net protein synthesis. In discussing this problem, however, we must keep in mind two lines of evidence which have been brought to attention in recent investigations. The first is the role of cytoplasmic RNA as a determinant factor in differentiation processes. The nuclear fibers of the lens continually produce albuminoid during the whole lifespan of the animal. This process can rightly be regarded as a differentiating one and it may require the presence of at least certain types of RNA. There is some evidence, indeed, that localized differentiating processes require the presence of special types of RNA at the place of differentiation. This would then be in agreement with the presence of albuminoid-linked RNA in the lens nucleus. Recent experiments also indicate that nucleic acids may function as polyelectrolytes which serve to activate certain enzyme processes and can, to a certain degree at least, be replaced by other nonspecific polyelectrolytes.