Effect of Hypophysectomy on Liver Nuclear Ribonucleic Acid Synthesis in Aging Rats

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Changes in RNA synthesis in liver nuclei were observed at different ages and after hypophysectomy and hormone replacement in female Sprague–Dawley rats. As determined by the incorporation of [^3H]UMP into an acid-insoluble product, RNA synthesis decreased by about 75% in intact rats from 6 months to 24 months of age. This decline with age was not observed in liver nuclei from 24-month-old rats that had been hypophysectomized at 12 months and maintained on a minimal hormone-replacement therapy. Thyroid hormones and somatotropin (growth hormone) had an additive effect on RNA synthesis in liver nuclei from these hypophysectomized rats. The same hormones had no significant effect on intact, age-matched rats. With advancing age, nuclei of intact rats had an increase in the pool of free RNA polymerase and an apparent decrease in the enzyme activity bound to nuclear chromatin. There was no change in total enzyme with age. In hypophysectomized, hormone-treated rats, free RNA polymerase activity decreased and chromatin-bound activity increased. There was no difference in total nuclear RNA polymerase activity between operated or intact rats. However, the ratio of the bound to the free activity was different. These results suggest that the ability of RNA polymerase to bind to chromatin may be involved in the age-related decrease in liver nuclear RNA synthesis of intact rats.

A decrease in the synthesis of protein and RNA of cells is one of the major changes reported in aging animals (Devi *et al.*, 1963; Barrows & Roeder, 1969; Mainwaring, 1968, 1969; Hrachovec, 1971; Geary & Florini, 1972; Higgins *et al.*, 1973; Castle *et al.*, 1978). Castle *et al.* (1978) reported that rat liver nuclear RNA synthesis increased from 4 to 6 months of age, then declined from 6 to 31 months. Several theories suggest that age-related changes in RNA and protein synthesis may be due to the cellular mechanisms regulating their synthesis (Orgel, 1970; Kormendy & Bender, 1971; Strehler *et al.*, 1971; von Hahn, 1971; Castle *et al.*, 1978). In young animals, hormone induction of specific transcription in target tissues has been shown to be a major aspect of this regulation (Tata & Widnell, 1966; Widnell & Tata, 1966; Higgins *et al.*, 1973; McCarty & McCarty, 1977). Widnell & Tata (1966) observed that liver nuclear RNA synthesis declined in rats after hypophysectomy, thyroidectomy or castration. RNA synthesis was restored by thyroid hormones, testosterone and somatotropin (growth hormone). The effects of these hormones were additive.

A recent series of studies have shown that the pituitary may secrete a substance(s) that has a dele-

terious effect on several functions of the body. When the pituitaries of 6–12-month-old rats were removed and the rats were tested at 16–24 months of age, a variety of functions were either arrested in their normal decline or were restored towards values observed in 2–6-month-old intact rats: minimal O_2 consumption (Denckla, 1975), xenograft rejection and colloidal carbon clearance (Bilder & Denckla, 1977), β -adrenergic-mediated relaxation of aortic strips (Parker *et al.*, 1978) and antibody production and plaque-forming ability (Scott *et al.*, 1979). In all examples, replacement with thyroid hormones was required, and in one instance somatotropin was also required to restore competence (Scott *et al.*, 1979). In view of these observations, the effects of age, long-term hypophysectomy and hormone replacement on rat liver nuclear RNA synthesis were investigated in female Sprague–Dawley rats. A possible mechanism for the observed changes is also considered.

Materials and Methods

Animals

Hypophysectomy of female Sprague–Dawley rats was done by Zivic–Miller Laboratories (Allison Park, PA, U.S.A.) by a standard transpharyngeal

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Table 1. *Schedule of hormone treatment of hypophysectomized rats*

The hormones were suspended in peanut oil and injected subcutaneously into the rats daily for 21 days before study. Control animals received a daily injection of an equal volume of peanut oil.

Hormone	Dose ($\mu\text{g/kg}$ body wt. per day)	
	Low	High
Tri-iodothyronine	2.5	8.3
Thyroxine	7.5	25.0
Corticosterone	300	300
Deoxycorticosterone	20	20

approach. After hypophysectomy, the rats were maintained on Purina Lab Chow, with approx. $\frac{1}{4}$ -normal concentration of major plasma cations (NaCl, 1.305 g/litre; NaH_2PO_4 , 1.17 g/litre; sodium citrate, 13.01 g/litre; KCl, 1.4 g/litre; magnesium acetate, 6.75 g/litre; CaCl_2 , 0.39 g/litre) and 3 mg of corticosterone acetate/litre added to the drinking water. The unoperated rats received Purina Lab Chow and water *ad libitum*. All rats were kept at Zivic-Miller until 4 weeks before use, and after receipt by us rats were maintained as described for 1 week. Before the experiments, hypophysectomized rats and age-matched intact rats received 21 daily subcutaneous injections of tri-iodothyronine, thyroxine, corticosterone and deoxycorticosterone as described in Table 1. In some experiments bovine somatotropin was added to the injection schedule at 300 $\mu\text{g/kg}$ body wt. per day. All animals were maintained on Purina Lab Chow and water during the 21-day injection period. Control rats that did not receive hormones were given daily injections of an equal volume of peanut oil.

Isolation of nuclei

The rats were killed by decapitation and the liver was placed within 30 s into ice-cold 50 mM-Tris/HCl (pH 7.4) that contained 5 mM-MgCl₂, 250 mM-sucrose and 50 μg of phenylmethanesulphonyl fluoride/ml (sucrose/TKM). The livers were washed with three changes of this buffer and nuclei isolated as described by centrifugation through sucrose density gradients (Bolla *et al.*, 1977). Nuclear DNA was determined by the diphenylamine method of Burton (1956) and the nuclei were counted with a haemocytometer.

RNA-synthesis assay

Activity of RNA polymerase on endogenous nuclear template (endogenous total activity) was assayed as previously described (Bolla *et al.*, 1977). Total nuclear RNA polymerase activity (chromatin-bound and free) was measured in liver nuclei from rats

of various ages and after various treatments. Liver nuclei (5×10^{12}) were suspended in 50 mM-Tris/HCl (pH 7.9)/2 mM-MgCl₂ and were disrupted by sonication (4×10 s at 50 W). The sonicated preparation was centrifuged at 5000g for 5 min to remove insoluble nuclear debris. RNA polymerase activity was then measured in a 200 μl reaction mixture containing 250 μg of calf thymus DNA as exogenous template, 20 μmol of $(\text{NH}_4)_2\text{SO}_4$, 20 μmol of Tris/HCl (pH 7.9), 1 μmol of MgCl₂, 0.1 μmol of *S*-adenosyl-methionine, 5 μg of pyruvate kinase, 0.1 μmol of phosphoenolpyruvate, 0.12 μmol each of ATP, GTP and CTP, 0.03 μmol of UTP, 5 μCi of [³H]UTP (specific radioactivity 37 Ci/mmol; New England Nuclear, Boston, MA, U.S.A.) and the sonicated preparation from 5×10^{12} nuclei. Activity of nuclear RNA polymerase not bound to the endogenous DNA template was measured in intact nuclei by the method of Lampert & Feigelson (1974). Nuclei were incubated in a 200 μl reaction mixture (Bolla *et al.*, 1977) containing 10 μg of actinomycin D/ml to inhibit endogenous RNA polymerase activity; 20 μg of poly[d(A-T)] was added as template. It was found that this concentration of actinomycin D inhibited 90% of endogenous nuclear RNA synthesis in all nuclei, but had no effect on transcription of the poly[d(A-T)] template. Incubations of nuclei without actinomycin D and poly[d(A-T)] were done to determine endogenous chromatin-bound activity. Total nuclear RNA polymerase activity was determined by incubating nuclei in the reaction mixture containing poly[d(A-T)], but without actinomycin D.

All assays were for 30 min, because RNA synthesis increased linearly during this time, after which a plateau was reached (results not shown). Activity of RNA polymerases I+III and II were determined on the basis of the differential sensitivity to α -amanitin (Jacob *et al.*, 1970).

Tri-iodothyronine, thyroxine, corticosterone and deoxycorticosterone were from Sigma Chemical Co., St. Louis, MO, U.S.A. Bovine somatotropin was prepared from 500 g of anterior pituitaries dissected from fresh frozen pituitaries. The tissue was homogenized in a Waring Blendor for 1 min and extracted for 30 min with 10 vol. of 50 mM-Tris base (pH 9.0). The extract was centrifuged at 25000g for 1 h and the resulting supernatant fractionated with $(\text{NH}_4)_2\text{SO}_4$ at 25–50% saturation (pH 7.5). The precipitate was dissolved in 2.5 litres of 3 M-urea/0.3 M-KCl/0.05 M-Tris base (pH 9.3–9.5) and filtered through an Amicon XM 100 membrane with 12.5 litres of the same buffer. The ultrafiltrate was dialysed on an Amicon PM 10 membrane with 5 mM-Tris base (pH 9.3–9.5) and concentrated to 200 ml. All preparative steps were at 5°C. The concentrated solution was placed nearest the cathode in a 50-chamber, 8 litre flat-bed-type isoelectric-focusing apparatus maintained at 5°C (Denckla, 1977). The sample solution

and the running solution contained 4M-urea and 0.1% Ampholines (pH 3.5–10; LKB). The cathode and anode solutions were prepared as described by Denckla (1977). The preparation was electrofocused for 10 days at 50 V/cm at 100 W (total voltage was 20000). The fraction focusing at pH 8.0–8.4 was precipitated with $(\text{NH}_4)_2\text{SO}_4$ to 50% saturation, and dissolved in and dialysed against 5 mM-Tris base (pH 9.0–9.5) for 24 h. The dialysed preparation was then electrofocused for 4 days at 40 V/cm in water containing 0.05% Ampholines and no urea. The fraction that was focused at pH 8.0–8.4 was precipitated with $(\text{NH}_4)_2\text{SO}_4$ to 50% saturation, dissolved in and dialysed against 5 mM-Tris base (pH 9.0). The final preparation was homogeneous after isoelectric focusing on polyacrylamide gels or electrophoresis in gels with sodium dodecyl sulphate. When it was injected at a dose of 1 mg/kg body wt. per day in peanut oil for 10 days, normal growth was obtained in 4-week-old hypophysectomized rats as compared with intact rats of the same age.

Results

RNA synthesis in liver nuclei from aging rats

The rate of RNA synthesis of nuclei from intact rats increased slightly from 4 to 6 months and then decreased. At 6 months of age the rate was approx. 4 times that at 24 months (Table 2). In order to determine if the decrease with age was due to increased ribonuclease activity, the nuclei were disrupted by sonication and incubated with ^3H -labelled rRNA prepared from *Escherichia coli* grown on ^3H -uridine (Gegenheimer *et al.*, 1977). After incubation at 27°C, hydrolysis of the radioactive RNA to acid-soluble material was determined by Millipore-filter assay (Bolla *et al.*, 1977). In agreement with others (Britton *et al.*, 1972; Castle *et al.*, 1978), ribonuclease

activity was insignificant and did not change with age.

The changes with age of the rates of both RNA polymerase types I+III and II closely paralleled the changes observed in total rates. After an initial rise from 4 to 6 months, there was a decline thereafter. The overall age-related change in RNA synthesis was therefore not due to a loss of a single major type of RNA. This is consistent with age-related changes in liver nuclear RNA synthesis in Fischer F344 rats reported by Castle *et al.* (1978) and Denckla (1975).

Age-related changes in RNA synthesis in long-term-hypophysectomized rats receiving hormone treatment

In these experiments 24-month-old rats that had been hypophysectomized at 12 months of age and 24-month-old intact controls were treated with various hormones (Table 1). Table 3 shows that treatment of intact rats with the hormones as described in Table 1 or with these hormones and somatotropin (300 µg/kg body wt. per day) had no effect on the rate of RNA synthesis when compared with age-matched untreated controls. However, when tri-iodothyronine, thyroxine, corticosterone, deoxycorticosterone and somatotropin were given to 24-month-old hypophysectomized rats, an increase in RNA synthesis was observed when compared with untreated hypophysectomized rats or treated and untreated intact rats. The difference between treated hypophysectomized rats and treated intact rats was significant ($P \leq 0.001$, Student's *t* test). The low and high doses of tri-iodothyronine and thyroxine increased RNA synthesis about 1.5- and 3-fold, respectively, compared with untreated hypophysectomized rats (Table 3, $P \leq 0.01$). Somatotropin had an additive effect and resulted in an additional 1.3-fold increase in RNA synthesis. The rate of RNA synthesis in liver nuclei of 24-month-old hypophysectomized rats that received all the hormones was not significantly different from the maximum rate found at any age in intact rats (6 months, Table 2). Since both activities of RNA polymerases I+III and II increased equally after hormone treatment of hypophysectomized rats, the increase was not due to selective stimulation of one type of polymerase.

Possible mechanism for age-related changes in RNA synthesis

The observed age- and hormone-treatment-related changes could be the result of: (1) differential loss of RNA polymerase from nuclei during isolation, (2) changes in enzyme specific activity, (3) decreased nuclear uptake of the enzyme, or (4) alterations in chromatin so that RNA polymerase cannot bind and initiate RNA synthesis. Since no age-related change in uptake of polymerase has been observed (Castle

Table 2. RNA synthesis in liver nuclei from rats of various ages

Total RNA-synthetic activity was determined as described in the Materials and Methods section. Activities of RNA polymerases I+III and II were determined by differential sensitivity to α -amanitin (Jacob *et al.*, 1970). All incubations were for 30 min at 27°C with saturating amounts of nucleoside triphosphates. Results are means $\pm$ S.E.M. ($n = 10$).

Age (months)	[^3H]UTP incorporated (pmol/ 10^{12} nuclei)		
	Total	I+III	II
4	418.5 $\pm$ 12.9	266.4 $\pm$ 19.6	126.5 $\pm$ 11.3
6	557.8 $\pm$ 25.9	321.3 $\pm$ 11.2	250.7 $\pm$ 14.6
12	426.5 $\pm$ 22.4	258.8 $\pm$ 19.3	169.2 $\pm$ 20.5
16	382.4 $\pm$ 8.6	232.4 $\pm$ 12.2	148.4 $\pm$ 10.9
18	276.5 $\pm$ 19.7	138.7 $\pm$ 9.4	126.2 $\pm$ 11.2
24	145.6 $\pm$ 10.4	82.4 $\pm$ 5.4	68.9 $\pm$ 8.5

Table 3. *RNA synthesis in liver nuclei from long-term-hypophysectomized rats*

Rats were hypophysectomized at 12 months of age (hypophysectomized) and maintained as described in the Materials and Methods section until they were 24 months old. Intact rats are 24-month-old unoperated controls. Hormones were given as described in Table 1. Somatotropin was given at 300 µg/kg body wt. per day. Total endogenous RNA polymerase activity was determined as described in the Materials and Methods section and in Table 2. Results are means ± S.E.M. (*n* = 10).

Treatment	[³ H]UTP incorporated (pmol/10 ¹² nuclei)		
	Total endogenous activity	I+III	I
Intact			
high	166.1 ± 18.6	96.2 ± 3.6	70.0 ± 11.4
low	159.4 ± 46.3	87.4 ± 2.9	70.9 ± 7.5
high + somatotropin	142.6 ± 36.1	86.4 ± 14.8	61.7 ± 11.2
Intact, no hormones	150.8 ± 28.2	98.9 ± 15.7	51.9 ± 18.1
Hypophysectomized, no hormones	155.0 ± 9.15		
Hypophysectomized			
high	488.7 ± 63.5*	256.5 ± 12.6	232.2 ± 42.9
low	289.7 ± 54.2*	195.2 ± 24.2	94.5 ± 29.1
high + somatotropin	677.0 ± 88.4*	342.8 ± 54.1	318.5 ± 42.0

et al., 1978; Gibas & Harman, 1970), it is unlikely that these changes arise from decreased nuclear uptake of RNA polymerase or precursor molecules to RNA synthesis. Several studies were conducted to investigate the other possibilities.

Table 4. *Total RNA polymerase activity in liver nuclei from various aged and long-term hypophysectomized rats*

Hypophysectomized rats were operated on at 12 months of age and maintained as described in the Materials and Methods section. Hormones were given as in Table 1. The dose of somatotropin given for the 21-day treatment schedule was 300 µg/kg body wt. per day. Calf thymus DNA was used as template for nuclear RNA polymerase in a 30 min incubation at 27°C. Saturating amounts of nucleoside triphosphates were used in each incubation. Specific radioactivity of [³H]UTP was 37 Ci/µmol. Results are means ± S.E.M. (*n* = 10).

Age (months)	Treatment	[³ H]UTP incorporated (pmol/mg of calf thymus DNA)
4	Intact	1513 ± 184
6	Intact	1518 ± 198
12	Intact	1510 ± 155
24	Intact	1404 ± 161
24	Intact + high tri-iodothyronine, thyroxine	1353 ± 218
24	Intact + low tri-iodothyronine, thyroxine	1235 ± 326
24	Hypophysectomized + high tri-iodothyronine, thyroxine	1551 ± 214
24	Hypophysectomized + low tri-iodothyronine, thyroxine	1531 ± 352
24	Hypophysectomized + high tri-iodothyronine, thyroxine and somatotropin	1500 ± 172

No age-related difference in total nuclear RNA polymerase activity was observed (Table 4). In addition, the total activity was not affected either by hormone treatment of intact rats or by hypophysectomy and subsequent hormone treatment. This suggests that the age-related changes in liver nuclear RNA synthesis are not due to either a loss of enzyme during isolation of the nuclei or an age-related decrease in the total activity of the enzyme, but rather these changes may involve an age-related restriction of the chromatin template to function in transcription.

Appreciable amounts of free RNA polymerase were observed in liver nuclei from intact rats (Table 5). The size of this pool was age-dependent; it was lowest in nuclei from 6-month-old rats and increased about 1.7-fold by 18 months (Table 5). There was no further increase from 18 to 24 months. The increase in free nuclear RNA polymerase activity was accompanied by a corresponding decrease in the concentration of chromatin-bound activity. As shown above (Table 4), there was no age-related change in total nuclear RNA polymerase activity (Table 5). The variation in the values of total RNA polymerase determined by the two different methods (Tables 4 and 5) may result from re-initiation of RNA synthesis on the calf thymus template, which may contain a large number of nicked regions. This would result in increased incorporation of [³H]UMP into shorter RNA molecules. Injection of 24-month-old intact rats with various hormones listed in Table 1 had no effect on the concentration of either free or chromatin-bound activity in the isolated liver nuclei when compared with untreated intact rats. Significant changes in free and chromatin-bound activity were observed, however, in nuclei isolated from hypo-

Table 5. Total, chromatin-bound and free RNA polymerase activity in liver nuclei from various aged rats and from long-term-hypophysectomized, hormone-treated rats

Hypophysectomy was at 12 months of age and the rats were maintained until they were 24 months old as described in the Materials and Methods section. Hormones were given as described in Tables 1 and 4. Chromatin-bound RNA polymerase activity was determined as described in the Materials and Methods section in the absence of actinomycin D and poly[d(A-T)]. Free RNA polymerase activity refers to the nuclear RNA polymerase activity not associated with the chromatin template. This activity was determined as described by Lampert & Feigelson (1974) in the presence of 10 μ g of actinomycin D/ml and 20 μ g of poly[d(A-T)]/ml. Results are means $\pm$ S.E.M. ($n = 10$).

Age (months)	Treatment	$[^3\text{H}]\text{UTP}$ incorporated (pmol/template)		
		Chromatin-bound	Free	Total
6	Intact	525.7 $\pm$ 14.6	396.5 $\pm$ 28.4	913.8 $\pm$ 48.2
12	Intact	473.5 $\pm$ 36.2	518.6 $\pm$ 41.6	954.9 $\pm$ 55.5
18	Intact	276.3 $\pm$ 47.7	642.7 $\pm$ 18.7	897.5 $\pm$ 24.8
24	Intact	212.0 $\pm$ 26.0	690.5 $\pm$ 45.7	908.9 $\pm$ 82.9
24	Intact, high tri-iodothyronine, thyroxine	234.6 $\pm$ 52.5	616.6 $\pm$ 54.5	898.8 $\pm$ 94.5
24	Hypophysectomized, tri-iodothyronine, thyroxine	497.9 $\pm$ 61.2	392.8 $\pm$ 58.6	908.2 $\pm$ 32.4
24	Hypophysectomized, tri-iodothyronine, thyroxine and somatotropin	636.2 $\pm$ 44.1	259.5 $\pm$ 45.1	874.5 $\pm$ 61.0

physectomized rats. Treatment of these rats with corticosterone, deoxycorticosterone and the high dose of tri-iodothyronine+thyroxine (Table 1) resulted in a 2-fold increase in chromatin-bound RNA polymerase activity and a corresponding decrease in free activity compared with treated intact 24-month-old rats. With the injection of somatotropin in addition to the other hormones, a further 1.3-fold increase in chromatin-bound activity occurred with a reciprocal decrease in free activity. Total nuclear RNA polymerase remained unaltered (Table 5). Thus after hormone treatment both the ratio and amounts of chromatin-bound and free RNA polymerase activity in 24-month-old hypophysectomized rats became comparable with the values found in 6-month-old rats.

To determine whether poly[d(A-T)] either competed with endogenous template for available nuclear RNA polymerase or caused a release of RNA polymerase from chromatin, liver nuclei from both intact and hypophysectomized hormone-treated rats were incubated in the standard reaction mixture with 5 μ Ci of $[^3\text{H}]\text{GTP}$ (specific radioactivity 25 Ci/mmol) substituted for radioactive UTP. Non-radioactive GTP was decreased to 0.03 μ mol and UTP increased to 0.12 μ mol in these assays. Synthesis was determined under these conditions in the presence and absence of 20 μ g of poly[d(A-T)]/200 μ l reaction mixture. Since poly[d(A-T)] will not direct the polymerization of $[^3\text{H}]\text{GTP}$ into acid-insoluble products, the only activity measured should be chromatin-bound activity unless poly[d(A-T)] interferes with this activity. Endogenous chromatin-bound activity in nuclei from variously aged and treated rats was not affected by poly[d(A-T)], and

the incorporation of $[^3\text{H}]\text{GTP}$ was identical in both the presence and the absence of poly[d(A-T)] (results not shown). This result is consistent with the report of Lampert & Feigelson (1974).

Discussion

The effects of age on liver nuclear RNA synthesis have been studied by others with varied results (Devi *et al.*, 1963; Gibas & Harman, 1970; Britton *et al.*, 1972; Castle *et al.*, 1978). In most studies young adults had more RNA synthesis than did old animals (Devi *et al.*, 1963, 1966; Britton *et al.*, 1972; Castle *et al.*, 1978), but Gibas & Harman (1970) did not observe any age-related change in nuclear RNA synthesis after 5 months of age. Their observations may have been influenced by the method of nuclear isolation and the assay conditions used. With methods comparable with those used in the present paper, Britton *et al.* (1972) found no age difference in total solubilized RNA polymerase activity from liver nuclei of mice. Their report is comparable with the present results. The age-related changes in RNA synthesis in liver nuclei from Sprague-Dawley rats reported in the present paper are in complete accord with the changes observed by Castle *et al.* (1978) in Fischer F344 rats, using similar methods.

It is possible that the observed changes with age are due to changes in precursor availability. However, no age-related changes in nucleotide uptake or metabolism (Castle *et al.*, 1978) have been observed. Since total RNA polymerase did not change with age when determined with calf thymus DNA or with endogenous chromatin plus poly[d(A-T)] as tem-

plates, the decrease in synthesis is not likely to be due to a decrease in total available RNA polymerase. These observations suggest that the age changes involve changes in the chromatin, not the polymerase. Further support for this model comes from the experiments with chromatin-bound and free polymerase at various ages. The increase in free polymerase with age and the decline in amount bound also suggests that the changes in chromatin may be of greater importance than changes in the enzyme. The data of Castle *et al.* (1978) are compatible with this model. Furthermore, the studies with α -amanitin indicate that there are no measurable changes with age in the ratio or total amounts of RNA polymerase types I+III and II and that the hormone injections did not affect the ratio of different types of polymerase.

Widnell & Tata (1966) and Tata & Widnell (1966) found that hypophysectomy of 6–8-week-old rats decreased RNA polymerase activity to the low values characteristic of our old intact or untreated hypophysectomized rats. Both thyroid hormones and somatotropin increased RNA polymerase activity of these young hypophysectomized rats to values close to those found in intact rats of the same age. The effects of the two hormones were additive. Intact rats (24 months old) had no response to the two different doses of thyroid hormones or to somatotropin. It was concluded that these older intact rats could not respond to these hormones. In 24-month-old hypophysectomized rats the higher dose of thyroid hormones produced a higher RNA polymerase activity than did the lower dose ($P \leq 0.01$), and the additional effect of somatotropin also produced a significant difference ($P \leq 0.01$). In contrast with 24-month-old intact rats, it was concluded that hypophysectomized rats of the same age could respond to both thyroid hormones and somatotropin. RNA polymerase represents the seventh thyroid-dependent (Bilder & Denckla, 1977; Denckla, 1974, 1975; Parker *et al.*, 1978) and the second somatotropin-dependent (Scott *et al.*, 1979) parameter that declines with age, appears to be associated with loss of peripheral tissue responsiveness to these hormones and is substantially restored by hypophysectomy. Previous measurements have shown no difference in plasma concentrations of thyroid hormones between treated intact or hypophysectomized rats (Parker *et al.*, 1978).

Liver nuclear RNA synthesis in female Sprague-Dawley and Fischer F344 rats apparently (Castle *et al.*, 1978) decreases with age, and this decrease is apparently not due to limitations of precursors or to enzymes of RNA synthesis, or to increased ribonuclease activity, but rather is due to template restriction. It is suggested that this restriction may involve alterations in the chromatin such that decreased

initiation of RNA synthesis occurs. This decreased initiation could account for the increased nuclear pool of free RNA polymerase.

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