Additive Effects of Thyroid Hormone, Growth Hormone and Testosterone on Deoxyribonucleic Acid-Dependent Ribonucleic Acid Polymerase in Rat-Liver Nuclei

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1. The stimulations of DNA-dependent RNA polymerase in isolated rat-liver nuclei by thyroid hormone, human growth hormone and testosterone are compared. 2. Single or multiple administrations of growth-promoting doses of tri-iodo-L-thyronine, human growth hormone and testosterone stimulate the Mg^{2+}-activated RNA-polymerase reaction in nuclei from thyroidectomized, hypophysectomized and castrated rats respectively. The magnitude of stimulation was proportional to the degree of enhancement of liver growth by each hormone. After a single injection, the latent period preceding the stimulation was 1, 2 and 10 hr. for growth hormone, testosterone and tri-iodothyronine respectively. The time-course of stimulation of enzyme activity and the synthesis of rapidly labelled nuclear RNA *in vivo* were also different for each hormone. 3. Growth hormone administration failed to stimulate the Mn^{2+}/ammonium sulphate-activated RNA-polymerase reaction. Thyroid hormone and testosterone, however, stimulated it but the effect was less pronounced and occurred several hours later than that observed for the Mg^{2+}-activated RNA-polymerase reaction. 4. In combination experiments, hypophysectomized or the thyroidectomized rats were given growth hormone or tri-iodothyronine in a single or repeated doses at levels that produced the maximum stimulation of Mg^{2+}-activated RNA-polymerase activity. Taking into account the different latent period for each hormone, a single administration of the second hormone caused an additional stimulation of the enzyme activity. Similar additive effects were observed in thyroidectomized–castrated rats after treatment with tri-iodothyronine and testosterone. The magnitude of the additional stimulation caused by the administration of the second hormone was compatible with the capacity of that hormone to promote liver growth in rats deprived of it. 5. It is concluded that, although these hormones have some similar effects, the regulation of nuclear RNA synthesis may be mediated via different routes for each hormone.

In the preceding paper (Tata & Widnell, 1966) we demonstrated that the DNA-dependent RNA polymerase (EC 2.7.7.6) in liver nuclei (Weiss, 1960) was stimulated after the administration of thyroid hormones to thyroidectomized rats. The stimulatory effect was detected independently for both the Mg^{2+}- and Mn^{2+}/ammonium sulphate-activated RNA-polymerase reactions (Widnell & Tata, 1964*a*). The effects of thyroid hormones on RNA synthesis *in vivo* (Tata & Widnell, 1966) and the response of RNA polymerase were compatible both with the nuclear control of cytoplasmic protein synthesis and the regulation of protein synthesis by thyroid hormones (Tata *et al.* 1963; Roodyn, Freeman & Tata, 1965). In recent years, similar evidence has been obtained about the important role of nuclear RNA synthesis in the biological action of other mammalian growth and developmental hormones such as growth hormone, testosterone and oestrogen (Korner, 1962, 1964; Talwar, Gupta & Gros, 1964; Williams-Ashman, Liao, Hancock, Jurkowitz & Silverman, 1964; Kochakian, 1965; Mueller, 1965). The mechanisms by which hormones affect RNA metabolism are still unknown, but in view of the many similarities of phenomena observed, the question arises whether different hormones act through the same pathway or perhaps even on some common cellular sites. A primary requirement for answering this question would be to select a tissue that would depend on two or more hormones for its growth and development.

The marked dependence of the liver of young animals on both growth hormone and thyroid hormones for its growth and metabolic activity is

well known (see Simpson, Evans & Li, 1949; Pitt-Rivers & Tata, 1959; Tata, 1964). Although the major growth-promoting action of testosterone is manifested in the prostate and seminal vesicles, the growth of other tissues of the body, including the liver, is accelerated in young castrated animals after the administration of testosterone (see Kochakian, 1965; Frieden, 1964). The anabolic action of each hormone is not impaired when they are administered in combination, in that additive effects may be observed at the whole body and tissue levels. The administration of thyroid hormone and growth hormone to hypophysectomized–thyroidectomized rats has been shown to cause additive increases in body weight (Simpson, Asling & Evans, 1950), and additive effects have been observed in the salivary gland, a tissue that responds to both thyroid hormones and testosterone (Schafer & Muhler, 1960; Raynaud, 1964).

Recent evidence from several Laboratories suggests that the activity of DNA-dependent RNA polymerase in isolated nuclei reflects the growth rate of the tissue (Hancock, Zelis, Shaw & Williams-Ashman, 1962; Weill, Busch, Chambon & Mandel, 1963; Gorski, 1964; Tsukada & Lieberman, 1964; Tata & Widnell, 1966; Pegg & Korner, 1965). Therefore the aims of the present work were, first, to determine whether the administration of growth hormone and testosterone *in vivo* caused a stimulation of DNA-dependent RNA-polymerase activity in isolated liver nuclei. Secondly, we have investigated whether additive effects could be observed when thyroid hormone and either growth hormone or testosterone were administered in combination.

In addition, the DNA-dependent RNA-polymerase activity of isolated rat-liver nuclei can be resolved into two reactions (Widnell & Tata, 1964*a*). One reaction, the product of which was similar to ribosomal RNA, is actived by Mg^{2+}, whereas the second reaction, activated by Mn^{2+} in the presence of ammonium sulphate, yields a product that is more DNA-like (Widnell, 1965). These two reactions respond to thyroid hormone *in vivo* at different time-intervals after hormone administration (Tata & Widnell, 1966) and it was therefore decided to determine whether comparable differences might be observed for growth hormone and testosterone. A preliminary communication of these results has been presented (Widnell & Tata, 1964*b*).

EXPERIMENTAL

Animals. Male Sprague–Dawley rats were used in all the experiments. Thyroidectomy and castration were performed surgically when the animals weighed about 40g., and hypophysectomy at 100g., 3–4 weeks before the experiments. All hormones were injected subcutaneously.

Hormones. 3,3′,5-Tri-iodo-L-thyronine was obtained from Glaxo Laboratories, Greenford, Middlesex, and dissolved for injection as described by Tata & Widnell (1966). Human growth hormone (0·7i.u./mg.) was obtained from Dr D. R. Bangham of the Division of Biological Standards at this Institute, and was dissolved in 0·15M-NaCl immediately before injection. Testosterone propionate was obtained from Paines and Byrne Ltd., Greenford, Middlesex, as a

Table 1. *Effect of hypophysectomy and castration, and of the administration,* in vivo, *of growth hormone to hypophysectomized rats, and testosterone propionate to castrated rats, on the specific activity of RNA polymerase in isolated liver nuclei*

Experimental details are given in the text. The specific activity of the Mg^{2+}-activated RNA-polymerase reaction is expressed as $\mu\mu$moles of [^{14}C]ATP incorporated into RNA/15min./mg. of DNA, and that of the $Mn^{2+}/(NH_4)_2SO_4$-activated RNA-polymerase reaction as $\mu\mu$moles of [^{14}C]ATP incorporated into RNA/45min./mg. of DNA. Each value is the average of two or three separate determinations on nuclei extracted from livers pooled from three rats.

Experimental animal	Expt. no.	Specific activity of RNA polymerase: Mg^{2+}-activated	Specific activity of RNA polymerase: $Mn^{2+}/(NH_4)_2SO_4$-activated
(*a*) Normal, unoperated	1	886	2920
	2	942	3380
Hypophysectomized	1	464	1990
	2	476	2610
Hypophysectomized, treated with 200μg. of human growth hormone 3hr. before death	1	700	1980
	2	849	2620
(*b*) Normal, unoperated	1	1240	3490
	2	1160	2680
Castrated	1	865	2750
	2	715	1790
Castrated, treated with 200μg. of testosterone propionate 16hr. before death	1	1205	3310
	2	1010	2400

solution in ethyl oleate, and was diluted in arachis oil before use.

Experimental techniques. The isolation of rat-liver nuclei, the determination of the two RNA-polymerase reactions and NAD pyrophosphorylase, and the assay of DNA, RNA and protein, were as described by Tata & Widnell (1966).

RESULTS

Effects of individual hormones. It was shown in the preceding paper (Tata & Widnell, 1966) that both the Mg^{2+} and Mn^{2+}/ammonium sulphate-activated RNA-polymerase reactions of isolated rat-liver nuclei were lower than normal in thyroidectomized animals and were stimulated at about 12hr. and 30hr. respectively after a single injection of tri-iodothyronine. Preliminary experiments also showed that the specific activity of the two RNA-polymerase reactions was lower in either hypophysectomized or castrated rats than in the unoperated controls. The results presented in Table 1(*a*) show that the specific activity of the Mg^{2+}-activated, but not that of the Mn^{2+}/ammonium sulphate-activated, RNA-polymerase reaction was stimulated 3hr. after a single injection of growth hormone to hypophysectomized rats. Fig. 1(*a*) shows the time-course of the stimulation after administration of the hormones; a small but reproducible stimulation was observed after 1hr. and the maximum at 3hr. No stimulation of the Mn^{2+}/ammonium sulphate-activated reaction was observed at any time-interval tested, whereas the rapidly stimulated Mg^{2+}-activated reaction had returned to the control value during the 48hr. time-period investigated.

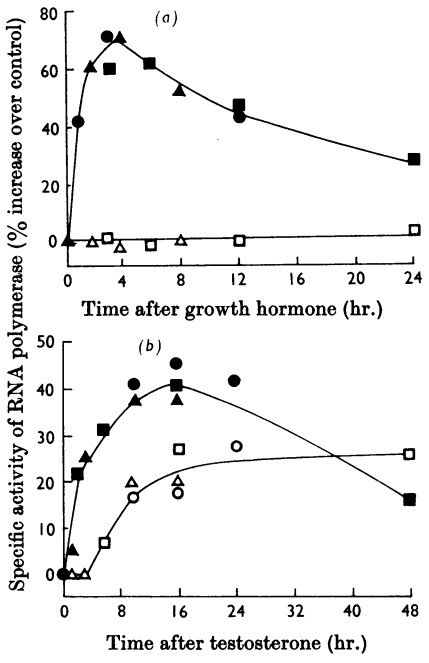

Fig. 1. Effect on the two RNA-polymerase reactions of isolated liver nuclei of a single injection of 200μg. of human growth hormone to hypophysectomized rats or 200μg. of testosterone propionate to castrated rats. The experimental details were as described in the text. ●, ▲ and ■, Mg^{2+}-activated RNA-polymerase reaction; ○, △ and □, Mn^{2+}/$(NH_4)_2SO_4$-activated RNA-polymerase reaction. Different symbols denote the values obtained from separate experiments. (*a*) Growth hormone administered to hypophysectomized rats; (*b*) testosterone propionate administered to castrated rats. Specific activities of the two RNA-polymerase activities were nearly the same as those in Tables 1, 2 and 5.

Table 1(*b*) shows that the specific activity of both RNA-polymerase reactions was stimulated 16hr. after a single injection of testosterone propionate to castrated rats. The time-course in Fig. 1(*b*) shows that, as with thyroid hormones (Tata & Widnell, 1966), the Mg^{2+}-activated reaction was stimulated before any change could be detected in the Mn^{2+}/ammonium sulphate-activated reaction. However, the latent period preceding the stimulation was shorter than that for thyroid hormones, in that the Mg^{2+}-activated reaction was stimulated within 2hr. and the Mn^{2+}/ammonium sulphate-activated reaction within 5hr. of hormone administration.

The stimulation of RNA polymerase as a function of the dose of growth hormone and testosterone administered to hypophysectomized and castrated rats respectively was comparable with the dose response for the effects on whole body growth (see Li, 1953; Kochakian, 1950). Fig. 2(*a*) shows that, after growth hormone administration, maximum stimulation of the Mg^{2+}-activated RNA-polymerase reaction was observed at a dose of 200μg. of growth hormone/100g. body wt.; no stimulation of the Mn^{2+}/ammonium sulphate-activated reaction was observed at any dose level. Fig. 2(*b*) shows the dose–response curves for the two RNA-polymerase reactions after testosterone propionate administration. For both the reactions the maximum stimulation was observed at a dose of 200μg. of testosterone propionate/100g. body wt.

As was also found for thyroid hormones (Tata & Widnell, 1966), the essential characteristics of both RNA-polymerase reactions, namely dependence on the presence of all four nucleoside triphosphates, DNA-dependence and the incubation time-course of the reaction, were unaffected by the endocrine status of the animals from which the liver nuclei were prepared. All three hormones were without effect on either RNA-polymerase reaction when added *in vitro* to nuclei obtained from hormone-deficient rats. No effects were observed on the specific activity of NAD pyrophosphorylase in the

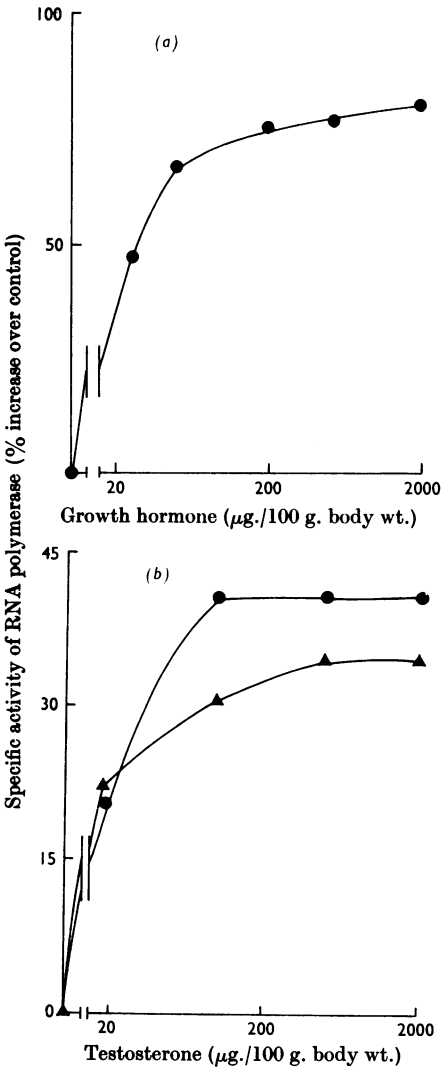

Fig. 2. Effect of the dose of human growth hormone administered to hypophysectomized rats, or testosterone propionate administered to castrated rats, on the two RNA-polymerase reactions of isolated liver nuclei. ●, Mg^{2+}-activated RNA-polymerase reaction; ▲, Mn^{2+}/$(NH_4)_2SO_4$-activated RNA-polymerase reaction. (*a*) Growth hormone administered to hypophysectomized rats 3hr. before killing; (*b*) testosterone propionate administered to castrated rats 16hr. before killing. Control values for untreated animals are shown in Tables 1, 2 and 5.

nuclear preparations from animals treated with the hormones *in vivo*, under conditions in which RNA polymerase was stimulated.

Additive effects of the hormones. In all the experiments described below on the effects of the hormones in combination, the dose of hormone administered, and the time-interval between the injection of the hormone and killing, were such that each hormone effected its maximum stimulation of RNA polymerase.

(*a*) Combination of thyroid hormone and growth hormone. The results of the first of the experiments involving a combination of hormones are shown in Table 2. The procedure consisted in giving daily injections for 8 days of either tri-iodothyronine or growth hormone to hypophysectomized rats. At the end of this period, these animals received a single injection of 25 μg. of tri-iodothyronine or 100 μg. of growth hormone 24 or 3hr. respectively before being killed. These doses and time-intervals were selected on the basis of the effects observed for each hormone independently [see Tata & Widnell (1966) for thyroid hormone; Figs. 1(*a*) and 2(*a*) for growth hormone]. Both chronic and acute treatment with either hormone caused a stimulation of the Mg^{2+}-activated RNA-polymerase reaction, and when the rats receiving chronic treatment with one hormone were subjected to acute administration of the other an additional stimulation of the enzyme was observed. Further, the magnitude of the stimulation of RNA polymerase caused by acute treatment with one hormone was essentially the same whether or not the animals had received chronic treatment with the other.

Table 3 shows that the administration of single injections of either tri-iodothyronine or growth hormone to hypophysectomized rats caused a stimulation of the Mg^{2+}-activated RNA-polymerase reaction. When the hormones were administered together as single injections to the same animal, the effect was essentially additive, whether the animals were killed 24, 42 or 45hr. after tri-iodothyronine, or 3 or 12hr. after growth hormone. Stimulation of the Mn^{2+}/ammonium sulphate-activated reaction was only observed 42 or 45hr. after tri-iodothyronine, and growth hormone was without effect on this reaction, in agreement with the findings reported in Table 1(*a*) and Fig. 1(*a*). Table 4 shows the results of a similar experiment in which thyroidectomized rats instead of hypophysectomized rats were used. Essentially the same results were obtained.

(*b*) Combination of thyroid hormone and testosterone. The results of two experiments in which thyroidectomized–castrated rats received chronic administration of tri-iodothyronine and testosterone propionate separately and in combination are shown in Table 5. The administration of either hormone caused a stimulation of both RNA-polymerase reactions, and when both hormones were administered the effect was essentially additive. As was observed with the combination of growth hormone and thyroid hormone (see Table 3), the

Table 2. *Effect of a single and of multiple doses of tri-iodothyronine and growth hormone on hypophysectomized rats as measured by the Mg^{2+}-activated RNA-polymerase reaction of isolated liver nuclei*

The specific activity of RNA polymerase was as expressed in Table 1. Three rats/group were used. Acute hormone treatment: a single injection of 25 μg. of tri-iodothyronine or 200 μg. of growth hormone was made at the time indicated before killing the animals. Chronic hormone treatment: 2 μg. of tri-iodothyronine or 25 μg. of growth hormone was injected daily for 8 days before killing the animals; the last dose of tri-iodothyronine was 24 hr., and growth hormone 6 hr., before killing. At the beginning of the experiment all animals weighed about 100 g., and chronic treatment was for 8 days. Body growth and basal metabolic rate (B.M.R.) were expressed relative to the untreated control animals on the day of killing.

Tri-iodothyronine treatment	Growth hormone treatment	Body wt. (% increase)	B.M.R. (% increase)	Specific activity of RNA polymerase	ΔRNA polymerase: Due to tri-iodothyronine (acute)	ΔRNA polymerase: Due to growth hormone (acute)
—	—	—	—	404		
Acute (24 hr.)	—	—	—	531	127	
—	Acute (3 hr.)	—	—	656		252
Chronic	—	6	49	578		
Chronic	Acute (3 hr.)	7	47	819		241
—	Chronic	13	11	658		
Acute (24 hr.)	Chronic	11	9	789	131	

Table 3. *Effect of the administration of single injections of tri-iodothyronine and growth hormone to hypophysectomized rats, separately and in combination, on the activity of the two RNA-polymerase reactions of isolated liver nuclei*

In both experiments hypophysectomized animals were used. The dose of tri-iodothyronine was 25 μg. and of growth hormone 200 μg.; the rats weighed about 100 g. The specific activity of RNA polymerase was expressed as in Table 1. Each value is the average of two determinations on nuclei from pooled livers (three rats/group).

	Time after single injection (hr.): Tri-iodothyronine	Time after single injection (hr.): Growth hormone	Specific activity of RNA polymerase: Mg^{2+}-activated	Specific activity of RNA polymerase: $Mn^{2+}/(NH_4)_2SO_4$-activated
Expt. 1	—	—	646	2040
	45	—	861	2710
	45	3	1245	2810
	—	3	955	2020
Expt. 2	—	—	556	2390
	24	—	732	2430
	24	12	1030	2410
	42	—	795	2920
	42	12	1090	3010
	—	12	876	2360

administration of a single injection of one hormone to rats maintained on multiple injections of the other caused a further stimulation of RNA polymerase (Table 6). Further, the magnitude of this additional stimulation of RNA polymerase by each hormone is compatible with their relative capacities to stimulate whole body growth.

Results obtained in this Laboratory have shown, both with thyroid hormone acting on rat liver [Tata & Widnell (1966) and Table 7] and oestrogen in the rat uterus (Hamilton, Widnell & Tata, 1965), that the stimulation of RNA synthesis *in vivo* preceded the stimulation of RNA polymerase in isolated nuclei. With growth hormone, however, there was an initial decrease in the specific activity of the rapidly labelled nuclear RNA followed by an increase at later time-intervals. The fall in specific activity of nuclear RNA preceded the stimulation of RNA polymerase, as also shown in Table 7. It is probable that there is not necessarily a correlation between RNA synthesis *in vivo* and the activity of RNA polymerase *in vitro*.

Table 4. *Effect of the administration of single injections of tri-iodothyronine and growth hormone to thyroidectomized rats, separately and in combination, on the activity of the two RNA-polymerase reactions of isolated liver nuclei*

The experimental details were as described in Table 3, except that thyroidectomized rats instead of hypophysectomized rats were used.

Time after single injection (hr.)		Specific activity of RNA polymerase	
Tri-iodothyronine	Growth hormone	Mg^{2+}-activated	$Mn^{2+}/(NH_4)_2SO_4$-activated
—	—	645	2090
24	—	987	2240
24	3	1385	2160
42	—	922	2895
42	3	1296	3010
—	3	959	2140

Table 5. *Effect of chronic administration of tri-iodothyronine and testosterone propionate on the activity of the two RNA-polymerase reactions of isolated liver nuclei*

The specific activity of RNA polymerase was expressed as in Table 1. At the beginning of the experiments all the operated animals weighed about 130g. Chronic treatment consisted of daily injections of the hormone for 12 days; the dose of tri-iodothyronine was 5μg./rat/day, and that of testosterone propionate 300μg. (Expt. 1) and 250μg. (Expt. 2)/rat/day. The last injections were given 24hr. before killing the animals. Nuclei from three pooled livers were used in each group.

		Chronic hormone treatment		Specific activity of RNA polymerase	
		Tri-iodothyronine	Testosterone propionate	Mg^{2+}-activated	$Mn^{2+}/(NH_4)_2SO_4$-activated
Expt. 1	Thyroidectomized rats	−	−	446	1650
		+	−	753	2420
		−	+	529	2130
		+	+	842	2850
	Unoperated control	−	−	871	2780
Expt. 2	Thyroidectomized castrated rats	−	−	412	1785
		+	−	761	2570
		−	+	493	2130
		+	+	819	2820
	Unoperated control	−	−	857	2750

Table 6. *Effect of chronic and acute administration of tri-iodothyronine and testosterone propionate to thyroidectomized–castrated rats on the activity of the two RNA-polymerase reactions of isolated liver nuclei*

The specific activity of RNA polymerase was expressed as in Table 1. At the beginning of the experiment all the animals weighed about 130g. Chronic treatment was the same as for Expt. 1 in Table 5. For acute treatment, the dose of tri-iodothyronine was 25μg. and that of testosterone propionate was 300μg.

		Specific activity of RNA polymerase	
Tri-iodothyronine treatment	Testosterone propionate treatment	Mg^{2+}-activated	$Mn^{2+}/(NH_4)_2SO_4$-activated
—	—	451	1825
Chronic	—	782	2810
—	Chronic	551	2350
Chronic	Chronic	858	3080
Chronic	Acute (10hr.)	829	3180
Acute (24hr.)	Chronic	878	2340

Table 7. *Increase in specific activity of rapidly labelled nuclear RNA* in vitro *and DNA-dependent RNA polymerase in isolated nuclei, as a function of time after a single injection of tri-iodothyronine or human growth hormone*

Tri-iodothyronine (18 μg./100g. body wt.) was administered to thyroidectomized rats and growth hormone (200 μg./100g.) to hypophysectomized rats. Six rats were used for each time-interval; of these, three were given 6 μc of [^{14}C]orotic acid/rat 10min. before killing and the other three were used for determining the specific activity of the Mg^{2+}-activated RNA-polymerase reaction. Values for hormone-treated animals are expressed relative to those found in the uninjected controls. Absolute values for uninjected (zero-time) controls were: rapidly labelled nuclear RNA: 29800 and 41500 counts/min./mg. of RNA for thyroidectomized and hypophysectomized rats respectively; Mg^{2+}-activated RNA polymerase: 540 and 485 $\mu\mu$c of [^{14}C]ATP incorporated into RNA/15min./mg. of DNA for the same two groups. Variations of values were within $\pm$10%.

Hormone	Time after injection (hr.)	Relative specific activity: Rapidly labelled nuclear RNA	Relative specific activity: RNA polymerase
Tri-iodothyronine	0	100	100
	4	115	99
	6	165	100
	10	195	105
	16	320	137
	24	305	162
Growth hormone	0	100	100
	1	70	136
	2	54	159
	3	96	178
	4	148	174
	6	138	179
	8	124	159

DISCUSSION

Hypophysectomy and thyroidectomy of young rats leads to a retardation or cessation of the growth of the liver and other tissues. The administration of growth hormone to hypophysectomized rats has been shown to stimulate liver growth (Simpson *et al.* 1949) and to increase the concentrations of liver RNA and protein (Geschwind, Li & Evans, 1950). Stimulation of amino acid incorporation into protein after growth hormone administration to rats has been demonstrated both *in vivo* (Korner, 1960) and in cell-free systems from the liver (Korner, 1959, 1961), and a stimulation of the incorporation of RNA precursors into RNA has been reported (Talwar, Panda, Sarin & Tolani, 1962; Iwamoto, Moriyama, Tetsuka & Miura, 1963; Talwar *et al.* 1964; Korner, 1964). The administration of small amounts of thyroid hormones (L-thyroxine and tri-iodo-L-thyronine) to thyroidectomized or hypophysectomized rats stimulates growth and increases the RNA and protein content of the liver (see Pitt-Rivers & Tata, 1959; Tata, 1964). Under conditions where whole body growth was stimulated, the administration of these hormones *in vivo* stimulated the incorporation of amino acids into protein both *in vivo* (Michels, Cason & Sokoloff, 1963) and in cell-free systems from the liver (Tata *et al.* 1963; Stein & Gross, 1962; Roodyn *et al.* 1965). We have also shown that these effects of the hormone on protein synthesis were preceded by a stimulation of nuclear RNA synthesis (Tata & Widnell, 1966). Castration of mice led to a decreased liver RNA/DNA ratio, which was restored to normal by testosterone administration (Kochakian & Harrison, 1962). It has also been found that administration of the hormone *in vivo* stimulated the incorporation of RNA precursors into RNA in the liver *in vivo* (Cantarow, Williams, Melnick & Patchkiss, 1958).

The present results, together with those of Tata & Widnell (1966), show that the Mg^{2+}-activated RNA-polymerase reaction of isolated rat-liver nuclei was stimulated by the administration *in vivo* of small doses of thyroid hormones, growth hormone and testosterone, and that the combination of thyroid hormone with either growth hormone or testosterone caused an additive stimulation. The Mn^{2+}/ammonium sulphate-activated RNA-polymerase reaction was stimulated by thyroid hormone and testosterone at time-intervals after hormone administration that were different from those observed for the Mg^{2+}-activated reaction [see Fig. 1(*b*); Tata & Widnell (1966)], and was not affected by growth hormone. The latent period for the response of either RNA-polymerase reaction

was different for growth hormone, testosterone (see Figs. 1*a* and 1*b*) and thyroid hormone (Tata & Widnell, 1966). The essential characteristics of each RNA-polymerase reaction, namely dependence on the presence of DNA and all four nucleoside triphosphates, and the quality of the RNA product, were unaltered by the endocrine status of the animals. The observation that no hormonal effect on NAD pyrophosphorylase could be detected suggests that the stimulation of RNA polymerase was not mediated by some general non-specific stimulation of nuclear activity.

Our results are generally in agreement with reports from other Laboratories on the effects of growth and developmental hormones on nuclear DNA-dependent RNA polymerase. Hancock *et al.* (1962) found that castration decreased, and testosterone administration *in vivo* stimulated, the DNA-dependent RNA-polymerase activity of an 'aggregate enzyme' preparation from prostatic nuclei; they also found that the effect of testosterone was less pronounced when the activity was measured in a medium of high ionic strength. Weill *et al.* (1963) have shown that oestradiol administration to chickens caused a stimulation of liver RNA polymerase, assayed in the presence of ammonium sulphate. Gorski (1964), however, was only able to detect stimulation of uterine RNA polymerase, at early time-intervals after the administration of oestrogen to ovariectomized rats, in the absence of ammonium sulphate. Pegg & Korner (1965) have also reported that they were able to detect a stimulation of enzyme activity in liver nuclei only in the absence of ammonium sulphate, after growth hormone administration *in vivo*. It has also been shown that the specific activity of the enzyme is greater in regenerating than in normal rat liver (Busch, Chambon, Mandel & Weill, 1962; Tsukada & Lieberman, 1964), and, together with hormonal effects, it can be said that the specific activity of RNA polymerase in various tissues provides an accurate reflection of the rate of tissue growth.

For several reasons, the effects of thyroid hormone, growth hormone and testosterone on RNA polymerase are compatible with the action of these hormones at the whole body level. First, the dose–response pattern of the stimulation of the Mg^{2+}-activated RNA-polymerase reaction is similar to that of the growth of the liver in the intact animal for each hormone. Secondly, the relative latent periods preceding the onset of the stimulation of the enzyme by the different hormones are consistent with those observed for whole body growth. Thirdly, the magnitude of the stimulation of RNA polymerase by the hormones administered, separately or in combination, is proportional to the relative capacity for promoting liver growth.

The mechanism of stimulation of RNA polymerase by hormones is not known but three possibilities could be invoked, separately or in combination, to account for the observations. The stimulation could be caused (*a*) by an allosteric interaction between the hormone and the enzyme, with the resulting conformational change that would increase enzyme activity (Monod, Changeux & Jacob, 1963), or (*b*) by some hormone-mediated effect on the DNA template, either increasing that fraction of the DNA active in RNA synthesis, or increasing the template efficiency of specific loci (Williams-Ashman *et al.* 1964), or (*c*) by the hormones inducing the synthesis of the RNA-polymerase protein, so that assay of the activity of the enzyme *in vitro* would reflect the quantity of the enzyme in the original tissue.

Reports from several Laboratories suggest that growth-promoting hormones, and even some hormones without any anabolic action, induce a general stimulation of RNA synthesis, particularly that of ribosomal RNA synthesis (Wool & Munro, 1963; Kenney & Kull, 1963; Talwar *et al.* 1964; Korner, 1964; Bransome & Chargaff, 1964; Moore & Hamilton, 1964; Wicks & Kenney, 1964; Tata & Widnell, 1966). In this context, the RNA-polymerase reaction in isolated rat-liver nuclei found in the present work to be stimulated by thyroid hormones, growth hormone and testosterone was the reaction that appeared to synthesize ribosomal RNA (Widnell, 1965). These observations focus attention on the possible importance of the synthesis of ribosomes in the mechanisms of action of growth-promoting hormones. At the same time, the additive effects suggest that, whatever the mechanisms by which thyroid hormone, growth hormone and testosterone stimulate RNA polymerase, the initial or fundamental site of action is probably different for each hormone.

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