Regulation of the Rate of Synthesis of Messenger Ribonucleic Acid by Growth Hormone

By A. KORNER

Department of Biochemistry, University of Cambridge

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Pituitary growth hormone regulates the growth rate and body weight of animals (Simpson, Evans & Li, 1949), and it probably does so by stimulating protein biosynthesis (Russell, 1955).

It has been shown (Korner, 1958, 1959, 1961*b*, 1962) that hypophysectomy and growth hormone treatment of rats can influence the amino acid incorporation into protein with liver cell-free systems, and that the hormonal control of protein synthesis can be explained in terms of its effect on the ability of ribosomes to assemble activated amino acids into polypeptide chains. Little effect of altering the concentration of circulating growth hormone by hypophysectomy or by injecting growth hormone into the rat was noted on the ability of the cell sap to activate amino acids and to attach them to s-RNA*; this may be because no change has occurred in the activity of these processes or that they are not limiting reactions under the conditions used. These conclusions from experiments with systems *in vitro* have been confirmed by work *in vivo* (Korner, 1960*a*), and it has also been shown that other hormones that control protein metabolism do so by altering the incorporating ability of ribosomes (Korner, 1960*b*, *c*, *d*).

The messenger hypothesis (Jacob & Monod, 1961), proposed to explain the genetic control of protein synthesis in bacteria, offered the possibility that the protein-anabolic hormones exert their control through an effect at the genetic level on the synthesis of m-RNA. This possibility was strengthened when various experiments demonstrated that the messenger hypothesis could be applied, in its essentials, to mammalian tissues. The present paper gives a number of results which strongly suggest that growth hormone acts on protein synthesis, and, thereby, on growth, by regulating the synthesis of m-RNA and of other types of RNA. A preliminary report on a part of the work has been published (Korner, 1963).

MATERIALS AND METHODS

Female albino rats were used. Hypophysectomy was performed by the para-pharyngeal method (Ingle &

Griffith, 1942), and completeness of removal of the pituitary gland was checked by observation at autopsy.

Ox growth hormone was prepared by the method of Wilhelmi, Fishman & Russell (1948) and was injected subcutaneously in aq. 0.9% NaCl. Control rats were injected with aq. 0.9% NaCl. Actinomycin D was dissolved in propylene glycol and administered by intraperitoneal injection, and again the control rats received the carrier by injection.

Polyuridylic acid was prepared from UDP by the method of Matthaei, Jones, Martin & Nirenberg (1962) by using the enzyme RNA polymerase extracted from *Micrococcus lysodeikticus*.

DL-[1-¹⁴C]Leucine (7 mc/m-mole), DL-[1-¹⁴C]phenylalanine (10.8 mc/m-mole) and [³H]orotic acid (106 mc/m-mole) were obtained from The Radiochemical Centre, Amersham, Bucks. The orotic acid was dissolved in dilute NaOH and injected intraperitoneally or, occasionally, intravenously into the femoral vein while the rat was under light ether anaesthesia.

Preparation of cell-free fractions. The rats were killed by decapitation and were bled. The livers were cut up in ice-cold 0.25M-sucrose solution containing medium M [tris buffer, pH 7.6 (20 mM), magnesium acetate (10 mM), KCl (100 mM), NaCl (40 mM) and mercaptoethanol (6 mM)], washed free of blood and lightly homogenized in a fresh solution of sucrose containing medium M. After removal of mitochondria, nuclei and debris by centrifugation at 10 000–15 000*g* for 10 min. at 0°, the supernatant was treated with one-ninth of its volume of ice-cold freshly dissolved 10% (w/v) sodium deoxycholate in 50 mM-tris buffer, pH 8.2. This deoxycholate-treated 10 000*g* supernatant was used (*a*) to prepare ribosomes by the method described by Korner (1961*a*), (*b*) to separate polysomes by the method of Wettstein, Staehelin & Noll (1963), except that the 2.0M- and 0.5M-sucrose solutions contained medium M rather than the salts described by Wettstein *et al.* (1963), and (*c*) to prepare the total ribonucleoprotein particles by centrifugation at 105 000*g* through medium M in 1M-sucrose. The ribosomes were suspended in medium M and washed free of deoxycholate by recentrifugation.

Cell sap was prepared by centrifugation of the 10 000*g* supernatant (without the addition of deoxycholate) at 105 000*g* for 2 hr. The cell sap was passed through a Sephadex G-25 column equilibrated with medium M to remove inhibitors (Mansbridge & Korner, 1963). The microsome pellet, when used to prepare RNA (see below), was suspended in 0.25M-sucrose solution containing tris buffer, pH 7.5 (20 mM), sodium dodecyl sulphate (2%, w/v) and bentonite.

Nuclei were separated from the liver homogenate by centrifugation at 600*g* for 6 min. The crude nuclear fraction was suspended in 0.25M-sucrose solution containing tris

* The abbreviations used are: s-RNA, transfer RNA; m-RNA, messenger RNA.

buffer, pH 7.5 (20 mM), filtered through gauze and washed by alternate centrifugations at 100g and 600g in 0.25M-sucrose solution containing tris buffer, pH 7.5 (20 mM). It was finally suspended in the same buffer containing sodium dodecyl sulphate (0.1%) and bentonite.

Gradient analysis of deoxycholate-treated 10 000g supernatant. The deoxycholate-treated 10 000g supernatant was analysed by sucrose-gradient-density centrifugation by layering 0.1 ml. of it on a linear 30–15% sucrose gradient containing medium M (without mercaptoethanol, which interfered with the extinction at 260 m μ in the presence of magnesium). A cushion of 0.5 ml. of 2M-sucrose solution containing medium M was put at the bottom of the tube. After centrifugation for 45 min. at 37 000 rev./min. drops were collected from the bottom of the tube and the extinctions at 260 and 320 m μ were determined. The reading at 260 m μ was corrected for the extinction of the sucrose in the sample and for that of the ferritin contaminant. The latter was carried out by subtracting 1.45 times the extinction at 320 m μ from the extinction at 260 m μ (Jackson, Munro & Korner, 1963).

Incubation. Incubation of ribonucleoprotein particles and cell sap in a final volume of 1 ml. and at 37° was carried out in medium M containing ATP (5 mM), GTP (0.1 mM) and DL-[1-¹⁴C]leucine (0.5 μ C). An ATP-generating system is not necessary for incorporation into ribosomes (Korner, 1961a), nor for polysomes. The reaction was stopped by the addition of 0.5N-HClO₄ containing leucine, and the protein was worked up for radioactivity counting as described by Korner (1961a). Each result reported is the mean of those obtained with three incubation tubes containing preparations from pooled livers of two or more rats. The results presented in the Tables and Figures are those of a typical experiment: similar results have been obtained in at least two other (and often in more) experiments.

Preparation and analysis of ribonucleic acid. RNA was extracted from ribosomes and microsomes suspended in sucrose-tris buffer, pH 7.5, by shaking with 2% sodium dodecyl sulphate and freshly redistilled phenol in the presence of bentonite to decrease ribonuclease breakdown of RNA (Hall & Doty, 1959). Then 0.1% sodium dodecyl sulphate was used to extract nuclear RNA, since this concentration is said not to extract DNA (Dingman & Sporn, 1962). Some of the nuclear RNA remained in the interphase layer and the yield of nuclear RNA was variable. Treatment of the interphase material with phenol at alkaline pH (Georgiev & Manteva, 1962) resulted in the release of RNA material.

The RNA in the aqueous layer was removed, the phenol layer was extracted again and the combined washings were treated once more with phenol. The RNA was precipitated with 2% (final concn.) sodium acetate buffer, pH 5.2, and 2½ vol. of ethanol, washed repeatedly with ethanol and redissolved in acetate buffer, and then reprecipitated with ethanol. It was finally dissolved in 10 mM-sodium acetate buffer, pH 5.2, containing NaCl (0.1M), and analysed by centrifugation for 4 hr. at 37 000 rev./min. on a linear 20–5% sucrose gradient containing sodium acetate buffer, pH 5.2 (10 mM) and NaCl (0.1M). Drops were collected from the bottom of the tube, the extinction was read at 260 m μ , and the radioactivity was assessed after precipitation and washing with ice-cold 0.5N-HClO₄ and 2% (v/v) acetic acid on to membrane filters which were heated at 60° for 1 hr. and counted in a Packard scintillation counter.

Estimation of protein and ribonucleic acid. Triplicate samples of ribonucleoprotein particles and of cell sap were used for protein estimation by the method of Lowry, Rosebrough, Farr & Randall (1951), and other triplicate samples were used for RNA estimation by the modification suggested by Fleck & Munro (1962) of the method of Scott, Fraccastoro & Taft (1956). The samples were precipitated with ice-cold 0.5N-HClO₄, nucleotides were removed by washing with ice-cold 0.5N-HClO₄ and the precipitate was hydrolysed for 1 hr. at 37° in 0.3N-NaOH. The pH was adjusted to 1 with 6N-HCl, and the extinction at 260 m μ of the acid-soluble fraction measured and the RNA content calculated by using an extinction coefficient of 34.2/mg./ml./cm. (Scott *et al.* 1956).

RESULTS

Polysomes. Table 1 shows the results of a typical experiment in which amino acid incorporation into protein was studied with ribosomes and polysomes prepared from livers of normal and hypophysectomized rats and of rats treated with growth hormone. As expected, ribosomes from hypophysectomized rats incorporated (per mg. of particle RNA) about half as much amino acid into protein as ribosomes from normal rats when incubated under identical conditions with the same concentration of the same preparation of cell sap treated with Sephadex. Rats given growth hormone showed the expected enhanced incorporating ability of their liver ribosomes (Korner, 1961b). Polysomes, prepared from the same deoxycholate-treated 10 000g supernatant material as the ribosomes, showed almost no difference in incorporating ability (per mg. of RNA) with different hormonal treatments of the rats (Table 1).

The yield of polysomes was, however, much less from the liver of hypophysectomized rats than from normal liver, as judged by RNA determinations of the yield of polysome pellet obtained from a known amount of deoxycholate-treated 10 000g supernatant from normal rat liver compared with the polysome yield from the same amount of deoxycholate-treated 10 000g supernatant from liver of hypophysectomized rats.

Fig. 1 shows the extinction at 260 m μ (corrected for the extinction due to ferritin contaminant; see the Materials and Methods section) of deoxycholate-treated 10 000g supernatants from normal and hypophysectomized rats after sucrose-gradient centrifugation. This shows clearly the lower yield of polysomes obtained from livers of rats deprived of their pituitary gland.

Treatment of the hypophysectomized rat with growth hormone raised the polysome yield.

A pellet of polysomes, unlike one of ribosomes, can be suspended without homogenization and does not require washing by centrifugation to remove deoxycholate, processes known to degrade poly-

some aggregates. It might be argued that the differences noted in incorporating ability between ribosomes from normal and hypophysectomized rats is simply a reflexion of greater damage to the active particles from hypophysectomized rats

Table 1. Amino acid incorporation into protein with ribosomes, polysomes and total ribonucleoprotein particles of liver of normal, hypophysectomized and growth-hormone-treated rats

Each of the three types of ribonucleoprotein particle was prepared from the same deoxycholate-treated 10 000g supernatant of rat liver as described in the Materials and Methods section. Approximately equal amounts of particle material were incubated with the same amount of the same preparation of cell sap. Hypophysectomy had been carried out 3 weeks previously and growth-hormone-treated rats had received 1 mg. 24 hr. and 1 mg. 12 hr. before the experiment.

Type of ribonucleoprotein particle	Treatment of rat	Radioactivity incorporated (counts/min./mg. of protein/mg. of particle RNA)
Ribosomes	None	815
	Hypophysectomy	400
	Hypophysectomy + growth hormone	770
Total ribonucleoprotein particles	None	1025
	Hypophysectomy	460
	Hypophysectomy + growth hormone	855
Polysomes	None	1160
	Hypophysectomy	1030
	Hypophysectomy + growth hormone	1100
	Hypophysectomy + growth hormone	1100

during resuspension and recentrifugation. This possibility can be discounted by the observation that preparations of the total ribonucleoprotein particles of the deoxycholate-treated 10 000g supernatant by centrifugation through 1M-sucrose show differences similar to the ribosomes, even though they do not have to be washed free of deoxycholate and are more easily suspended than ribosomes (see Table 1).

The results of Table 1 and Fig. 1 suggest strongly that the total ribonucleoprotein-particle population of the 10 000g supernatant from hypophysectomized rats contains less polysomes (ribosomes attached to m-RNA) and more free monomeric and dimeric ribosomes not attached to m-RNA than a similar preparation from normal rat liver. When the ability of the total particle population to incorporate labelled amino acids into protein is assayed (i.e. with ribosome or total ribonucleoprotein-particle preparations), the specific activity is less with preparations from hypophysectomized rats because more inactive ribonucleoprotein particles are present. When this ability is assayed with polysomes, concentrated from the total ribonucleoprotein-particle population, little difference is noted (in counts/min./mg. of particle RNA) since most of the inactive particles have not been centrifuged down into the polysome pellet. The small differences in specific activity that are found probably reflect more contamination of the polysome pellet from hypophysectomized rats with inactive monomers than is the case with the preparation from normal rats.

Addition of polyuridylic acid and ribonucleic acid. The argument outlined suggests that hypophysectomy of the rat has resulted in a fall in the ability of rat-liver ribosomes to attach themselves to m-RNA or that less m-RNA is available in the rats deprived of their pituitary gland. The former possibility is seen to be untenable by the results summarized in Table 2. The addition of polyuridylic acid to ribosomes prepared from liver of hypophysectomized rats resulted in at least as much, and usually more, stimulation of incorporation of phenylalanine than when normal ribosomes were used.

Similar results were obtained when rat-liver RNA was added to ribosomes in that the system from hypophysectomized rats was certainly not less able to respond to stimulation than that from normal rats. The results show that growth hormone is not altering the ability of ribosomes to attach themselves to any available m-RNA. The alternative possibility, that less m-RNA is present in preparations from hypophysectomized rats, remains.

Effect of actinomycin and growth hormone on amino acid incorporation. Treatment of rats with actinomycin inhibits amino acid incorporation into

Fig. 1. Sucrose-gradient-centrifugation analysis of deoxycholate-treated 10 000g supernatant of liver of normal (Δ) and hypophysectomized (●) rats. Experimental details are given in the Materials and Methods section.

ribosomal protein (Korner & Munro, 1963), in part because of inhibition of the synthesis of m-RNA. Table 3 summarizes results of experiments in which rats were injected with both growth hormone and actinomycin.

When 1 mg. of growth hormone was given 12 hr. before the rat was killed, there was a modest stimulation of amino acid incorporation into protein of ribosomes of normal rats and of rats treated with 50 μ g. of actinomycin. Rats given 200 μ g. of actinomycin showed both a greater inhibition of incorporation *in vitro* and greater percentage stimulation of incorporation by growth hormone. Rats given 500 μ g. of actinomycin showed greater inhibition of incorporation but a smaller stimulation with growth hormone than those given 200 μ g. Although inhibition of incorporation increases with increasing dose of actinomycin, the percentage stimulatory effect of growth hormone was greater

in the rats given moderate doses of actinomycin than in rats given either small or large doses of the drug. In animals given moderate amounts of actinomycin, protein synthesis was inhibited sufficiently to demonstrate the growth hormone effect yet not inhibited to such a degree that the animal was unable to respond vigorously to growth hormone.

The extent of growth hormone stimulation of amino acid incorporation in actinomycin-treated rats is also dependent on the time allowed to elapse between administration of the antibiotic and assay of the effect on the cell-free system. Table 4 shows that the incorporation obtained after injection of 250 μ g. of actinomycin decreases with time after injection for some hours and then reaches a plateau. Growth hormone stimulation (measured as a percentage increase) occurs to a greater extent during the middle times studied: again too small or too great an inhibition masks the effect of the growth hormone.

Labelling of ribonucleic acid. Table 5 summarizes the results of labelling nuclear and cytoplasmic RNA with [3 H]orotic acid in normal and hypophysectomized rats and in similar rats given a single injection of 1 mg. of growth hormone some hours before death. Very marked changes can be seen. Hypophysectomized rats incorporated orotic acid into both nuclear and cytoplasmic RNA to a lower extent than normal rats, and a single injection of growth hormone into hypophysectomized rats greatly raised the incorporation into nuclear RNA and into microsomal RNA. Substantial increments of orotic acid incorporation into RNA were also obtained when normal rats were given growth hormone.

Actinomycin treatment of the rat caused the expected (Meritz, 1963; Franklin, 1963) inhibition of RNA synthesis. When conditions of actinomycin treatment (dose and time) were such that a substantial growth hormone effect on stimulating ribosomal amino acid-incorporating ability could be expected (cf. Tables 3 and 4), it was also possible to show a growth hormone stimulation of RNA synthesis in the actinomycin-treated animals (Table 5). Growth hormone stimulated the synthesis of both nuclear and microsomal RNA of actinomycin-treated rats.

Experiments of the type described in Table 5 were carried out with different pulse times of orotic acid and this resulted in marked differences in the relative distribution of radioactive RNA between nuclear and cytoplasmic fractions. In addition, a higher specific radioactivity of nuclear RNA was found when pure preparations of nuclei were achieved. Finally, variations in extraction procedure (see the Materials and Methods section) used for nuclear RNA resulted in differences of yield of

Table 2. *Effect of hypophysectomy and growth hormone treatment on the polyuridylic acid stimulation of phenylalanine incorporation*

Experimental details are given in the Materials and Methods section.

Source of ribosomes	Polyuridylic acid (25 μ g.)	Radioactivity incorporated (counts/min./mg. of protein/mg. of particle RNA)
Normal rats	—	480
	+	1390
Hypophysectomized rats	—	260
	+	1210
Hypophysectomized rat given 1 mg. of growth hormone 12 hr. previously	—	425
	+	1260

Table 3. *Effect of growth hormone and actinomycin treatment of rats on ribosomal amino acid incorporation in vitro*

Experimental details are given in the Materials and Methods section.

Treatment of rat	Growth hormone (1 mg. 12 hr. before expt.)	Radioactivity incorporated (counts/min./mg. of protein/mg. of ribosomal RNA)
None	—	950
	+	1170
50 μ g. of actinomycin 12 hr. before expt.	—	710
	+	850
200 μ g. of actinomycin 12 hr. before expt.	—	390
	+	790
500 μ g. of actinomycin 12 hr. before expt.	—	210
	+	280

nuclear RNA from experiment to experiment. Despite these variations a clear effect of growth hormone stimulation of orotic acid incorporation into nuclear and microsomal RNA could be seen at each time studied.

Sucrose-gradient-centrifugation analysis of samples of the microsomal RNA obtained from normal, hypophysectomized, actinomycin-treated and growth-hormone-treated rats given [³H]orotic acid are shown in Fig. 2. The radioactivity of microsomal RNA after a 30 min. pulse with orotic acid is mainly in the 6–18 s region and is not associated with the major extinction peaks: this 6–18 s RNA has some of the characteristics of m-RNA (Munro & Korner, 1962). With longer intervals after the injection of [³H]orotic acid more of the radio-

activity appeared in the 19 s RNA and in the 28 s ribosomal structural RNA. Some radioactivity is present in the 4 s soluble RNA at all time-intervals.

Hypophysectomy of the rat decreased the incorporation of orotic acid into all types of RNA including m-RNA. Treatment of the hypophysectomized rats with growth hormone resulted in a clear increase in labelling of the RNA in all regions including the m-RNA region. A similar but smaller effect of increasing the labelling of m-RNA was noted when growth hormone was given to normal rats.

Actinomycin treatment stopped almost all orotic acid incorporation except into 4 s RNA: this can be accounted for entirely by the turnover of the terminal three nucleotides of s-RNA (Meritz, 1963; Franklin, 1963). Treatment of actinomycin-treated rats, both normal and hypophysectomized, with growth hormone resulted in increased labelling in all RNA fractions including that usually considered to be m-RNA.

Sucrose-gradient analyses of the nuclear RNA gave results shown in Fig. 3. The *E*_{260 mμ} patterns revealed peaks at about 33, 19 and 6 s similar to those obtained by Sporn & Dingman (1963) and Hiatt (1962), and the radioactivity was spread throughout the gradient in a fashion similar to that

Table 4. *Effect of time after actinomycin treatment on response of rat to growth hormone stimulation of amino acid incorporation into protein*

Experimental details are given in the Materials and Methods section.

Time after injection of 250 μg. of actinomycin (hr.)	Growth hormone (1 mg. 12 hr. before death)	Radioactivity incorporated (counts/min./mg. of protein/mg. of ribosomal RNA)	Percentage increase of incorporation with growth hormone treatment
0	—	825	28
	+	1055	
4	—	635	39
	+	880	
7	—	390	110
	+	785	
14	—	230	91
	+	440	
24	—	280	34
	+	375	

Table 5. *Incorporation of [³H]orotic acid into liver nuclear and microsomal ribonucleic acid of normal and hypophysectomized rats given actinomycin and growth hormone (40 min. pulse)*

Experimental details are given in the Materials and Methods section.

Actinomycin 200 μ g. 12 hr. before expt.)	Growth hormone (1 mg. 12 hr. before expt.)	Radioactivity incorporated (counts/min./ $E_{260m\mu}$)	
		Nuclear RNA	Microsomal RNA
Normal rats			
—	—	1550	1680
—	+	2400	2520
+	—	270	245
+	+	760	545
Hypophysectomized rats			
—	—	920	1020
—	+	2170	2210
+	—	165	245
+	+	480	390

Fig. 2. Sucrose-gradient-centrifugation analysis of liver microsomal RNA of rats given [³H]orotic acid 45 min. before death. —, *E*_{260 mμ}; ·····, radioactivity (counts/min.). (a) Normal rat; (b) normal rat given 1 mg. of growth hormone 12 hr. before; (c) hypophysectomized rat; (d) hypophysectomized rat given 1 mg. of growth hormone 12 hr. before; (e) normal rat given 250 μg. of actinomycin 12 hr. before; (f) normal rat given 250 μg. of actinomycin and 1 mg. of growth hormone 12 hr. before. Experimental details are given in the Materials and Methods section.

Fig. 3. Sucrose-gradient-centrifugation analysis of liver nuclear RNA of rats given [^3H]orotic acid 45 min. before death (see legend of Fig. 2 for details).

found by Hiatt (1962). Again it was clear that growth hormone stimulated incorporation of orotic acid into all types of RNA and could continue to do so even in actinomycin-treated rats.

DISCUSSION

The results of the present experiments, taken together, indicate that growth hormone exerts its effects on protein synthesis and growth by stimulating the synthesis of m-RNA.

That growth hormone exerts a stimulatory effect on the synthesis of liver RNA has been known for some time. Hypophysectomy results in a fall in the RNA content of rat liver, and the administration of growth hormone reverses this fall (Reid, O'Neal, Stevens & Burnop, 1956; Di Stefano & Diermeier, 1959). Talwar, Panda, Sarin & Tolani (1962) reported a marked stimulation of incorporation of ^{32}P into fractions of rat-liver RNA after injection of growth hormone. These results, and the present ones, show a clear effect of growth hormone on the synthesis of the bulk RNA of the cell.

The present results, however, go further than this in indicating that growth hormone can also control the rate of synthesis of m-RNA. This is the simplest explanation for the finding that growth hormone regulates the incorporating ability of the total ribonucleoprotein-particle population but not of the polysomes isolated from the total population, and that the number of polysomes obtained is

dependent on the growth hormone treatment of the rat. That growth hormone can stimulate amino acid incorporation even in actinomycin-treated rats also suggests a hormone effect on m-RNA synthesis, for actinomycin acts on protein synthesis by the inhibition of m-RNA synthesis. It could still be argued, however, that the hormone stimulates some part of the protein-synthetic system other than m-RNA synthesis.

The experiments on the labelling of RNA with [^3H]orotic acid, however, directly confirm the idea, since growth hormone treatment of the rat can stimulate the incorporation of orotic acid, not only in normal and hypophysectomized rats, but also in actinomycin-treated rats. Moreover, the pattern of labelling of RNA, revealed by the sucrose-gradient-centrifugation analysis of microsomal RNA, makes it clear that growth hormone not only stimulates the incorporation of orotic acid into structural RNA of the ribosomes, but also can stimulate orotic acid incorporation into the rapidly labelled m-RNA.

Hiatt (1962) has been unable to detect any rapidly labelled 6–18 s RNA in the cytoplasm from liver cells, but such RNA has been detected, not only during the experiments reported in the present paper, but also by Munro & Korner (1962).

The extinction patterns obtained from nuclear RNA were similar to those obtained by other workers (Hiatt, 1962; Sporn & Dingman, 1963). The correlation between size of RNA and function is not absolutely clear, but it seems probable that the 33s and 19s RNA have arisen from nuclear ribosomes. Most of the RNA of nuclei became labelled a short time after the injection of [^3H]orotic acid and it is not easy to assign the name 'messenger RNA' to any of the nuclear RNA fractions. It is clear, however, that treatment of rats with growth hormone stimulated orotic acid incorporation into all types of RNA whereas hypophysectomy and actinomycin treatment curtailed incorporation.

Increasing the rate of synthesis of RNA could stimulate protein synthesis by increasing the availability of either m-RNA or of active ribosomes; it is possible to envisage the supply of either being limiting in some cells under some conditions although it is generally assumed that availability of m-RNA is the more usual controlling condition because of its greater lability. It seems clear that, at least in liver, the first effects of administration of growth hormone on protein synthesis are mediated through the enhanced synthesis of m-RNA rather than of ribosomal RNA.

Growth hormone appears to be a general protein-anabolic hormone in that most tissues grow under its influence, albeit to different extents (Greenbaum & Young, 1953). Hypophysectomized animals, though smaller than intact ones, appear to be

reasonably well proportioned. Although there is no evidence of excessive synthesis of particular proteins in growth-hormone-treated animals, there is evidence that in muscle the proportion of protein synthesized under the influence of growth hormone differs somewhat from that in normal muscle (Gray & Young, 1954). If growth hormone exerts its effects by increasing the rate of synthesis of m-RNA, it seems that it must increase the rate of synthesis of the m-RNA molecules for, at any rate, most proteins, and must keep these increased rates of synthesis reasonably in step with each other. Alternatively one must postulate that some other mechanism operates to maintain homeostasis in the face of an unbalanced stimulation of synthesis of a few types of m-RNA by growth hormone.

Actinomycin has been shown to exert its effects on RNA synthesis by masking the guanine bases of the primer DNA (Goldberg & Rabinowitz, 1962), thus stopping polymerization of all DNA-primed RNA. It is very unlikely that growth hormone is acting at the same point for this would imply that growth hormone, given to normal rats, unmasks primer DNA which would result in the production, not of more of the same kinds of proteins, but of new types of protein. The results suggest that when actinomycin has blocked some of the DNA primer, but not all of it, growth hormone stimulation of some other part of the RNA-synthesizing system can make use of the unblocked primer DNA more efficiently than is possible in the absence of the hormone.

A reasonable, but not unique, candidate for the site of action of growth hormone is the RNA polymerase enzyme itself, but we can, as yet, produce no experimental evidence in favour of this suggestion; however, a reasonable speculation (by no means the only one) is that RNA polymerase is an allosteric protein (Monod, Changeaux & Jacob, 1963) and that its activity can be altered by conformational alteration of the enzyme brought by the binding to it of some specific metabolite(s). It is unlikely that a molecule as large as growth hormone itself (ox growth hormone has a mol.wt. of about 45 000) can be an allosteric effector, but there is evidence that suggests that the growth hormone, isolated from pituitary glands, is not identical with the hormone *in vivo*, so that some smaller derivative of it may be the actual allosteric effector. An alternative possibility is that growth hormone does not act directly on the RNA polymerase but causes the secretion or synthesis of a small metabolite that acts as the effector.

Preliminary results suggest that insulin, which is known to have a protein-anabolic action (Korner & Manchester, 1960; Manchester & Young, 1961; Randle, 1963), and which can influence ribosomal incorporating ability (Korner, 1960*b*, *c*), also acts

by stimulating the synthesis of RNA (see also Wool, 1962; Wool & Munro, 1963). In addition Tata (1963) and Widnell & Tata (1963) have shown that the activity of thyroid hormone is inhibited by actinomycin, and Liao & Williams-Ashman (1962) have presented evidence that testosterone can influence the synthesis of m-RNA (see also Wilson, 1962). The influence of oestrogens on RNA synthesis has also been demonstrated (Ui & Mueller, 1963; Noteboom & Gorski, 1963; Talwar & Segal, 1963). It may be that the hormones that influence protein biosynthesis each do so by different means, but it is at least possible that they all act by means of an allosteric effect of the hormones themselves, or of some metabolite secreted as a result of their action, on RNA polymerase. The differences between the different protein-anabolic hormones may lie in the differences in their ability to influence this enzyme in different tissues: testosterone, for example, may act specifically on accessory sexual tissue because the structure of RNA polymerase in these cells may differ from that of the same enzyme in different tissues and may be changed only by the particular chemical structure of testosterone. Growth hormone, which is able to influence protein synthesis in most body tissues, may have a more general control of growth because the allosteric effector controlled by growth hormone can influence the activity of RNA polymerase of most tissues.

SUMMARY

1. The ability of rat-liver ribosomes to incorporate amino acid into protein *in vitro* depends on the concentration of circulating growth hormone in the rat, yet little change was observed in the incorporating ability of polysome preparations from liver of normal, hypophysectomized and growth-hormone-treated rats. The yield of polysomes was, however, shown to vary with the amount of circulating growth hormone.

2. Polyuridylic acid stimulation of phenylalanine incorporation was as great, or greater, with ribosomes from liver of hypophysectomized rats than with normal ribosomes.

3. Growth hormone was able to exert its stimulatory effects on the protein-synthetic ability of ribosomes, and on the number of polysomes, when injected into actinomycin-treated rats.

4. Growth hormone treatment stimulated orotic acid incorporation into nuclear and microsomal RNA of hypophysectomized and normal rats and in actinomycin-treated rats. At least part of the RNA synthesized as a result of growth hormone treatment is messenger RNA.

5. It was concluded that the rate of synthesis of rat-liver RNA, including messenger RNA, is regulated by growth hormone, and that the effects

of growth hormone on protein synthesis and growth can be explained in terms of its control of messenger RNA synthesis.

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