2. There are two periods in which liver nucleic acids (DNA, cytoplasmic RNA and nuclear RNA) show maximal rates of labelling. The first occurs at 2 days after birth and the second period at 8–10 days after birth.

3. Long-term experiments on the retention of [^{14}C]orotic acid in DNA after a single injection show that some of the DNA initially labelled at 2 days is transient, whereas all the radioactivity incorporated at 8 days after birth is retained for long periods.

4. Zone electrophoresis and zone centrifugation have resulted in a crude fractionation of liver of the 2-day-old animal into haematopoietic cells and liver-cell nuclei, and the labelling of the DNA of these fractions shows that about 70 % is accounted for by liver-cell DNA. The haematopoietic-cell DNA is more highly labelled but accounts for the minor proportion of the total labelled DNA, and for the transient material.

5. There are two periods during growth of the neonatal rat liver in which DNA and RNA are rapidly synthesized.

This work was financed by grants from the Medical Research Grants Committee of the University of Western Australia, and the National Health and Medical Research Council of Australia. The authors are indebted to Mr A. Buxton, Miss Robin Weaver and Miss Jennifer Cooke for technical assistance.

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Biochem. J. (1964), **91**, 565

Effect of Growth Hormone on Ribonucleic Acid Metabolism

3. NATURE AND CHARACTERISTICS OF NUCLEAR SUBFRACTIONS STIMULATED BY HORMONE TREATMENT*

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(Received 3 September 1963)

Talwar, Panda, Sarin & Tolani (1962) reported that growth hormone causes a significant increase in the incorporation *in vivo* of [^{32}P]orthophosphate into the nuclear and microsomal fractions of rat-liver RNA.

Experimental results from various sources indicate that most, if not all, of the cellular RNA is synthesized in the nucleus. A fraction of RNA (messenger RNA), having a rapid turnover rate and having the biological properties of 'transcribing' the genetic information and the base ratios corresponding to the species DNA, has been described in bacteria (Gros *et al.* 1961; Brenner, Jacob & Meselson, 1961). Hiatt (1962) has isolated a rapidly labelled RNA from the rat-liver nuclei.

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Similarly, ribosomal RNA-DNA hybrid fractions have been described (Yankofsky & Spiegelman, 1962*a, b*), implying the nuclear synthesis of ribosomal RNA. In higher organisms, Sibatani, de Kloet, Allfrey & Mirsky (1962) reported the isolation of four fractions of RNA from thymus nuclei. Besides the nuclear transfer RNA and ribosomal RNA, they were able to separate three other RNA fractions from the animal-tissue nuclei. A fraction of RNA that is extractable in 1M-sodium chloride is linked to the DNA strands. Sibatani *et al.* (1962) also described the isolation of a nucleolar fraction having the same base ratios as the DNA.

Since in our studies growth hormone has been shown to stimulate the synthesis of nuclear RNA, it was decided to determine the type of fraction concerned in this effect, and more particularly to discover if it can be defined as a messenger RNA.

The present paper describes the specific radioactivity and other characteristics of the three sub-fractions of nuclear RNA from normal and growth-hormone-treated animals.

MATERIALS AND METHODS

Animals. Albino rats, A.I.I.M.S. strain or Institut Pasteur strain (derived from Wistar strain), were used for all studies. They were either female 'plateaued' 6-month-old rats or 3-4-week-old litter mates.

Labelled compounds and reagents. [6-¹⁴C]Orotic acid (specific radioactivity 5.2 mc/m-mole) was obtained from the Commissariat de l'Energie Atomique, France; [³²P]orthophosphate (specific radioactivity 0.5 mc/ml. in iso-osmotic 0.9% NaCl solution) containing phosphate buffer, pH 7.4 (total solids 15 mg./ml.), was supplied by the Atomic Energy Establishments, Trombay, India.

Actinomycin D was kindly given by Dr M. Cohn. Pancreatic ribonuclease, deoxyribonuclease and lauryl sulphate were purchased from Sigma Chemical Co. Teepol was a product of Burmah Shell. All other reagents were E. Merck A.-G. or British Drug Houses Ltd. AnalaR grade.

Growth hormone. Bovine growth hormone (NIH-GH-B5) and ovine growth hormone (NIH-GH-S4) were kindly supplied by Dr A. E. Wilhelmi as a gift from the Endocrinology Study Section, National Institutes of Health.

A weighed quantity of the hormone was suspended in sterile 0.9% NaCl solution (10 mg. in 1 ml.) and dissolved with a minimal addition (about 0.05 ml.) of 0.1N-NaOH. The pH of the final solution was between 7.4 and 8. Usually about 0.5 ml. of the solution was utilized for intraperitoneal injection in an animal.

Hormone treatment. Rats were kept without food overnight. Then 3.0 mg. of the hormone/100 g. body wt. was injected intraperitoneally in a volume of 0.5 ml. unless specified otherwise. Control animals received 0.5 ml. of 0.9% NaCl. Then, 5 hr. after administration of the hormone, labelled precursor ([6-¹⁴C]orotic acid or [³²P]orthophosphate) was injected. The dose of [³²P]orthophosphate used was 100 µC/100 g. body wt. and that of [6-¹⁴C]orotic acid was 6 µC (0.5 mg.)/100 g. body wt.

Preparation of liver nuclei. After 60, 90 or 120 min. of precursor incorporation, the animals were decapitated and the livers were perfused with chilled 0.25M-sucrose through the hepatic vein. For the density-gradient centrifuging, the nuclei were prepared as described by Allfrey & Mirsky (1957). For certain other experiments, the tissue was homogenized in 4 vol. of 0.25M-sucrose in a Potter-Elvehjem-type homogenizer fitted with a Teflon pestle. The sediment after centrifuging for 10 min. at 700g in an International refrigerated centrifuge (rotor 269; 2000 rev./min.) was resuspended in 4 vol. of 0.25M-sucrose and sub-layered with an equal volume of 0.34M-sucrose. Centrifuging for 10 min. at 700g yielded a loosely packed sediment of nuclei that was separated by carefully siphoning off the supernatant.

Nuclear ribonucleic acid fractions. Three RNA fractions were prepared from the nuclei by the method of Sibatani *et al.* (1962). These fractions were:

(a) Nuclear transfer RNA and ribosomal RNA (fraction N-1).

(b) An RNA extracted with DNA in 1M-NaCl and precipitated, with 2 vol. of ethanol, together with DNA strands (fraction N-2).

(c) The nucleolar RNA (fraction N-3) obtained by phenol treatment of the residue left after the extraction of fractions N-1 and N-2, and precipitated from the aqueous phase with ethanol. The extraction procedure for this fraction was slightly modified. The suspension of the nucleolar fraction in 0.05M-phosphate buffer, pH 6.85, was shaken with 0.05 vol. of 2.5% (w/v) bentonite, and lauryl sulphate was added to give a final concentration of 0.25%. The mixture was shaken for a few minutes, and an equal volume of 90% (w/v) phenol was added. The mixture was stirred for 1 hr. at room temperature. The aqueous extracts were collected, and the phenol was added. The mixture was stirred for 1 hr. at room temperature. The aqueous extracts were collected, and the phenol phase was washed once again with phosphate buffer. Potassium acetate was added to give a final concentration of 2% (w/v) and the RNA was precipitated with 2 vol. of cold ethanol. The precipitate was washed twice with ethanol to remove phenol, the ethanol in turn being removed with ether.

The 'nucleolar II fraction' reported by Sibatani *et al.* (1962) to be present in the phenol phase was not obtained in our experiments.

Cytoplasmic RNA. The supernatant obtained after separation of the nuclei was processed to obtain the cytoplasmic RNA. The method employed was identical with that used for the preparation of nucleolar RNA.

Determination of radioactivity. Radioactivity of RNA was determined as follows:

(a) Where [6-¹⁴C]orotic acid was the precursor, 0.1 ml. of the RNA solution was precipitated with 2 ml. of cold 5% (w/v) trichloroacetic acid and counted after filtration on a Millipore filter (Millipore Filter Corp., Bedford, Mass., U.S.A.) in a Nuclear-Chicago low-background gas-flow counter.

(b) In experiments where [³²P]orthophosphate was the precursor, the RNA fraction was hydrolysed with 0.3N-KOH for 18 hr. at 37°. The mixture was acidified with 60% (v/v) perchloric acid to pH 1, and centrifuged at 1600g for 10 min. The supernatant was neutralized by the addition of KOH, left in the cold for 1 hr. to permit the potassium perchlorate to settle and centrifuged at 700g for 10 min. A sample (0.1 ml.) of the supernatant was plated uniformly on a planchet, on which a drop of Teepol had been applied, and counted with a Tracerlab Beta-Scintillation Detector connected to a Tracerlab Compu/Matic Scaler.

Quantitative determination of ribonucleic acid. (a) RNA determinations were done spectrophotometrically by measuring the extinction at 260 mµ. An extinction of 0.49 was given by 16 µg. of standard hydrolysed yeast RNA/ml.

(b) In experiments where the orcinol colour reaction of the RNA subfraction was used, Ceriotti's (1955) modification of Meijbaum's procedure was employed.

Sucrose-density-gradient fractionation. The ethanol-precipitated RNA fractions were dissolved in 10 mM-acetate buffer, pH 5, containing NaCl (0.1M). A portion (1 ml.) of this solution was layered over a linear gradient of sucrose from 5 to 20% (Gros *et al.* 1961) and centrifuged in a swinging bucket (rotor SW25) of the Spinco ultracentrifuge model L at 45000g (23000 rev./min.) for 11 hr. A hole was made in the cellulose nitrate tubes with a sharp needle, and the contents were collected serially in small

Table 1. *Distribution of radioactivity on short-pulse labelling of RNA in livers of normal and growth-hormone-treated rats*

[³²P]Orthophosphate (100 µc/100 g. body wt.) was injected intraperitoneally into normal and growth-hormone-treated (3 mg./100 g. body wt.) rats. After 15 min., RNA was prepared from nuclear and cytoplasmic fractions of the liver and hydrolysed by alkali, and the resulting nucleotides were separated, by the methods described in the text. The counts/min./mg. of RNA are given as means ± s.e.m. of the results of four experiments. The results given in the Table are for nuclear RNA; no significant radioactivity was detected in the cytoplasmic RNA or in the ribonucleotides obtained from it.

	Counts/min./ mg. of RNA	Sp. radioactivity of nuclear RNA (counts/min./µmole of nucleotide in RNA)			
		2',3'-AMP	2',3'-GMP	2',3'-CMP	2',3'-UMP
Control rats	294.5 ± 45.4	118	29	64	90
Growth-hormone-treated rats	523 ± 59.9	208	103	120	200
Increase (%)	77.6	76	255	87	122

tubes. About 8–10 drops were taken in each tube for measurement of the radioactivity and 1 drop for the determination of the extinction at 260 mµ.

Actinomycin treatment. Actinomycin D in 0.9% NaCl solution was injected intraperitoneally at a dose of 1.5 mg. (or 3 mg./kg. body wt. (0.3 mg. or 0.6 mg./rat) 15 min. before the administration of the hormone (or 0.9% NaCl).

Determination of base ratios. RNA 2',3'-nucleotides obtained (as described in the section on the measurement of [³²P]orthophosphate radioactivity) by alkaline hydrolysis were concentrated by evaporation to a suitable volume, so as to contain about 1 mg. of nucleotides/0.1 ml. of the solution. A sample (0.02 ml.) of this concentrate was deposited 14 cm. from one end on a Whatman paper no. 3MM, moistened with 20 mM-citrate buffer, pH 3.5. The paper was inserted in a rheo-ionophoretic apparatus, which is a hanging-strip electrophoresis apparatus (Macheboeuf, Rebeyrotte & Brunerie, 1951; Williams, Pickels & Durrum, 1955). Rheo-ionophoresis was carried out at an applied voltage of 750 v (14.1 v/cm. paper length) for 6 hr. One drop of picric acid, which has an ionophoretic mobility similar to that of 2',3'-UMP, was generally used as a visual indicator in each run. The paper was air-dried and the spots of the four nucleotides were marked with the help of a Mineralight ultraviolet lamp. The spots were generally well resolved with a minimum distance of 1½–2 cm. between any two of them. The spots were cut out, their radioactivities determined by flattening them in planchets and the nucleotides eluted by overnight immersion in 5 ml. of 0.01 N-HCl for 2',3'-UMP, 2',3'-AMP and 2',3'-CMP, and in 5 ml. of 0.1 N-HCl for 2',3'-GMP. The eluate was centrifuged at 1600g for 10 min. The purity of the nucleotides was judged by the ratios of the extinction at (a) 250 and 260 mµ, (b) 280 and 260 mµ, and the quantity of the nucleotide was estimated from the $E_{260}^{1\text{cm.}}$ values by utilizing the standard molar extinction coefficients given by Beaven, Holiday & Johnson (1955).

Determination of deoxyribonucleic acid. This was done by the diphenylamine reaction of Dische (1955).

RESULTS

Early effects of the hormone on ribonucleic acid biosynthesis. In previous experiments (Talwar *et al.* 1962) the incorporation of radioactive precursor was allowed for a period of 60 min., at the end of

Table 2. *Specific radioactivity of the subfractions of liver nuclear ribonucleic acid in normal and growth-hormone-treated rats*

Bovine growth hormone (3 mg./100 g. body wt.) (or 0.9% NaCl) was injected intraperitoneally into female 'plateaued' rats 5 hr. before the administration of 1 mg. of [6-¹⁴C]orotic acid (12 µc) to each rat. The incorporation time was 90 min. Nuclear fractions were prepared as described in the text. The results were obtained with pooled tissues from three rats in each group.

Nuclear subfraction	Sp. radioactivity (counts/min./ $E_{260}^{1\text{m}\mu}$ unit)		
	Normal rats	Growth- hormone- treated rats	Increase (%)
N-1 (ribosomal RNA and transfer RNA)	22	50	127
N-2 (RNA linked to DNA)	163	389	138
N-3 (nucleolar RNA)	367	1341	265

which the microsomal and nuclear fractions of RNA were markedly labelled. In the hormone-treated animals both these fractions had a higher specific radioactivity than the control group. For shorter periods of labelling (15 min.) the incorporation of precursor was restricted to the nuclear fraction only, no radioactivity being detectable in the cytoplasmic fractions of RNA at this time (Table 1). The stimulation of the biosynthesis of RNA caused by growth hormone was localized in the nuclear fraction in the initial stages.

Turnover rates of the various nuclear ribonucleic acid fractions. Scholtissek & Potter (1960) reported a linear rate of precursor incorporation into rat-liver nuclear RNA for 2 hr. By utilizing [6-¹⁴C]orotic acid as precursor, we found at the end of 90 min. (Table 2) that the nucleolar fraction (N-3) had the highest specific radioactivity, then came the nuclear fraction (N-2) extractable with DNA, and finally the fraction N-1 (composed of the bulk

of transfer RNA and ribosomal nuclear RNA), the specific radioactivity of which was much lower. Treatment with growth hormone further magnified the differences between fraction N-3 and the two others, though it markedly stimulated the rate of labelling of these fractions.

Density-gradient fractionation of the nuclear sub-fractions. Since fractions N-2 and N-3 exhibited significantly higher rates of labelling under normal conditions than did fraction N-1, these were examined further on a sucrose-density gradient, with the hope of locating also the individual peak (or peaks) undergoing maximal stimulation by hormone treatment.

N-2 fractions. The DNA-associated RNA separated in a single sharp peak with a low sedimentation constant (Fig. 1). The radioactivity migrated

Fig. 1. Sedimentation diagram of nuclear subfraction N-2 on a sucrose-density gradient. ●, $E_{260\text{ m}\mu}^{1\text{ cm.}}$; ○, radioactivity (counts/min.). [$6\text{-}^{14}\text{C}$]Orotic acid was used as precursor. The incorporation time was 120 min. Experimental details are given in the text. Liver preparations from growth-hormone-treated rats gave a sedimentation profile identical with that of the control group.

in a parallel manner with the ultraviolet-absorption pattern, giving a single sharp peak. Treatment with growth hormone did not modify the physical characteristics of this fraction nor the radioactivity distribution pattern. Since this fraction of RNA had not been treated with phenol, it could be a ribonucleoprotein, perhaps contaminated with a deoxyribonucleoprotein, although the fraction had been treated with deoxyribonuclease. The radioactive material was sensitive to treatment with alkali or ribonuclease, and was resistant to deoxyribonuclease (Table 3).

The molar ratios of the pyrimidine and purine nucleotides in this fraction are given in Tables 4 and 5.

The nucleotide composition of this fraction remains almost unaltered by treatment with growth hormone. The specific activity of the labelled precursors reveals further an equivalence between the purines and pyrimidine nucleotides.

Table 3. Effects of various treatments on the radioactivity retained in the N-2 fraction

A sample (0.1 ml.) of N-2 RNA preparation (see the text) labelled with [$6\text{-}^{14}\text{C}$]orotic acid was taken in each tube. At the end of the treatment described 2 ml. of cold 5% (w/v) trichloroacetic acid was added to each tube and each precipitate was filtered on a Millipore disk and washed extensively with 5% trichloroacetic acid. The preparation from growth-hormone-treated rats was from a separate experiment.

Treatment	Radioactivity (counts/min.)	
	Normal rats	Growth-hormone-treated rats
None	296	687
Deoxyribonuclease (10 $\mu\text{g./ml.}$) for 15 min.	264	664
Ribonuclease (50 $\mu\text{g./ml.}$) for 15 min.	11	31
N-NaOH (0.1 ml./ml.) for 5 min.	3	3
Heating at 80° for 5 min. and ribonuclease (50 $\mu\text{g./ml.}$) for 15 min.	16	29

Table 4. Molar ratios of nucleotides in N-2 and N-3 subfractions of nuclear ribonucleic acid isolated from normal and growth-hormone-treated rats

Experimental details are given in the text. The results were obtained with pooled tissues from six animals in each group.

Nuclear sub-fraction		Percentage molar composition of nuclear RNA				A/U ratio	G/C ratio	(A + U)/(G + C) ratio	Purines/pyrimidines ratio
		2',3'-AMP	2',3'-GMP	2',3'-CMP	2',3'-UMP				
N-2	Normal rats	22.2	22.4	29.8	25.6	0.87	0.74	0.91	0.80
	Growth-hormone-treated rats	21.9	23.0	30.0	25.2	0.87	0.76	0.89	0.80
N-3	Normal rats	19.6	29.6	28.2	22.5	0.87	1.05	0.73	0.97
	Growth-hormone-treated rats	19.8	28.8	31.1	20.2	0.98	0.92	0.67	0.95

Table 5. *Percentage distribution of radioactivity, expressed as apparent molar base ratios of nucleotides in the N-2 and N-3 subfractions of newly synthesized ribonucleic acid isolated from normal and growth-hormone-treated rats*

Experimental details are given in each group. The results were obtained with pooled tissues from six animals in each group.

Nuclear sub-fraction	Percentage molar composition of newly synthesized RNA				A/U ratio	G/C ratio	(A + U)/(G + C) ratio	Purines/pyrimidines ratio
	2',3'-AMP	2',3'-GMP	2',3'-CMP	2',3'-UMP				
N-2	Normal rats	27.6	17.5	25.5	29.3	0.94	0.68	1.32
	Growth-hormone-treated rats	27.5	16.4	25.8	30.3	0.91	0.63	1.36
N-3	Normal rats	23.6	26.1	25.2	25.0	0.94	1.03	0.94
	Growth-hormone-treated rats	23.1	24.8	29.7	22.4	1.03	0.83	0.83

N-3 nucleolar ribonucleic acid. The nucleolar RNA fraction (N-3) was polydisperse and on sucrose-density-gradient fractionation it gave a broad peak with perhaps two discernible subfractions as major components (Fig. 2a). The radioactivity closely followed the extinction. Treatment with growth hormone induced a marked rise in the specific radioactivity of one of the major components of this fraction (Fig. 2b).

The molar nucleotide composition of this fraction (N-3) is represented in Tables 4 and 5. This fraction is richer in the proportion of guanine and cytidine nucleotides than is the N-2 fraction.

Treatment with growth hormone seems to increase the proportion of cytidine-and-adenine-rich RNA components.

Cytoplasmic ribonucleic acid. Fig. 3 represents the density-gradient fractionation pattern of cytoplasmic RNA from liver of normal (Fig. 3a) and growth-hormone-treated (Fig. 3b) rats. [6-¹⁴C]-Orotic acid was the labelled precursor and the incorporation time *in vivo* was 150 min. It is evident that the specific radioactivity of the various cytoplasmic RNA subfractions is elevated after treatment with growth hormone.

Inhibition by actinomycin of nuclear ribonucleic acid synthesis. A single dose of intraperitoneal actinomycin D caused a marked inhibition of the synthesis of rat-liver nuclear RNA, as has also been reported by Meritz (1963). The stimulation of the synthesis of nuclear RNA caused by growth hormone is completely arrested by actinomycin (Table 6).

DISCUSSION

Subcellular site of the initial growth-hormone effect on ribonucleic acid biosynthesis. In studies by Talwar *et al.* (1962) growth hormone was observed to cause a significant increase in the specific radioactivity of the nuclear and microsomal fractions of RNA. Short-pulse-labelling experiments have demonstrated that the stimulation occurs initially in the nuclear fractions (Table 1). With longer

incorporation times *in vivo* the microsomal fractions also became highly labelled. These results indicate that the origin of the stimulatory process is in the nucleus. Further, the fractions of RNA stimulated by the hormone have an interconnexion with the microsomal RNA.

Nature of the nuclear subfractions and the growth-hormone effect. Fraction N-1 is primarily composed of the nuclear transfer RNA and ribosomal RNA, and has a relatively low turnover rate (Table 2). Its role seems to be chiefly in the synthesis of nuclear proteins. The ability of the nuclei to synthesize proteins from radioactive amino acid precursors has been reported by Frenster, Allfrey & Mirsky (1961). This fraction is only moderately stimulated by the treatment with growth hormone.

Fraction N-2 is extracted with DNA proteins in 1M-sodium chloride. The evidence that it has a polyribonucleotide nature is: (1) a positive orcinol reaction; (2) fairly high labelling when [6-¹⁴C]-orotic acid is used as precursor; (3) sensitivity of the radioactive material to ribonuclease or alkali although deoxyribonuclease has no action (Table 3).

In a sucrose-density gradient fraction N-2 sediments in a single sharp peak of fairly low sedimentation constant, perhaps of about 2-4s (Fig. 1). The radioactivity migrates in a parallel manner to the extinction at 260 mμ. There is no information available on the nature or biological role of this fraction. It may represent some chains of RNA loosely attached to DNA strands. From its sensitivity to ribonuclease, it does not appear to be a true hybrid (Schildkraut, Marmur, Fresco & Doty, 1961). Treatment with growth hormone yields a fraction that has a sedimentation pattern identical with that of the N-2 fraction from untreated rat liver. The molar base composition of this fraction of RNA from livers of normal rats is identical with that of hormone-treated rats (Tables 4 and 5), as also is the percentage distribution of radioactivity in the four nucleotides, i.e. apparent molar base ratio of the newly synthesized RNA (Table 5). The nature of RNA of this fraction thus remains un-

changed by the hormone action. The specific radioactivity does undergo an increase that could signify an increase in the number of such strands in the hormone-treated group.

The N-3 fraction, which is considered to be nucleolar RNA (Sibatani *et al.* 1962), is heterogeneous and sediments as a broad double peak in a sucrose gradient (Fig. 2*a*). This fraction has a high turnover rate (Table 2). Treatment with growth hormone markedly stimulates incorporation into a part of the RNA comprising the broad double peak (Fig. 2*b*). The influence of the hormone is maximal on this fraction. The nature of nucleolar

Fig. 2. Sedimentation diagram of nuclear subfraction N-3 from the livers of (a) normal and (b) growth-hormone-treated rats, on a sucrose-density gradient. ●, $E_{260}^{1\text{ cm.}}$; ○, radioactivity (counts/min.). [$6\text{-}^{14}\text{C}$]Orotic acid was used as precursor. The incorporation time was 120 min. Experimental details are given in the text.

Fig. 3. Sedimentation diagram of cytoplasmic RNA, from the livers of (a) normal and (b) growth-hormone-treated rats, on a sucrose-density gradient. [$6\text{-}^{14}\text{C}$]Orotic acid was used as precursor. The incorporation time was 150 min. ●, $E_{260}^{1\text{ cm.}}$; ○, radioactivity (counts/min.).

Table 6. *Inhibition by actinomycin D of the nuclear ribonucleic acid synthesis*

Ovine growth hormone (1 mg./100 g. body wt.) was injected intraperitoneally 5 hr. before [^{32}P]orthophosphate (100 μC /100 g. body wt.). The incorporation time was 30 min. Nuclei were prepared by sublayering of 0.34 M-sucrose as described in the text. Actinomycin was injected 15 min. before the administration of growth hormone (or 0.9% NaCl).

Actinomycin dose	Sp. radioactivity (counts/min./mg. of nuclear RNA)	
	Expt. 1 0.3 mg.	Expt. 2 0.6 mg.
Treatment		
None	3543	2345
Growth hormone	5826	3188
Actinomycin	1343	618
Growth hormone + actinomycin	1049	700

RNA is still controversial. Sirlin (1960), Sirlin & Jacob (1962) and Fitzgerald & Vinijchaikul (1959) have suggested that the nucleolus would be an active site of RNA synthesis from the nucleolus-associated chromatin, but others have ascribed a storage role to the nucleolus, RNA synthesized from the extranucleolar region accumulating in the nucleolus before passage to the cytoplasm (Goldstein & Micou, 1959; Pelling, 1959). Perry (1962) has observed that the nucleolus would be the site for the synthesis of rapidly sedimenting heterogeneous RNA which, on subsequent 'chase' incubation, passes to the cytoplasm and is the precursor of the cytoplasmic ribosomal RNA. Our experimental results are consistent with this observation. The growth hormone primarily stimulates the synthesis of the nucleolar fractions of RNA that are the precursors of the microsomal RNA fractions (Fig. 3). There is sufficient evidence to indicate that the hormone action results also in increased capability of protein synthesis. The incorporation of labelled amino acids in liver *in vivo* and *in vitro* is much decreased in hypophysectomized rats. The administration of growth hormone partially restores the incorporation (Korner, 1960). By using a cell-free system Korner (1959) showed that, though the activation of amino acids and their transfer with transfer RNA is normal in hypophysectomized rat-liver preparations, the subsequent steps in protein synthesis occurring at the microsomal level are impaired. The current concepts of protein synthesis involve messenger RNA together with the ribosomal RNA and transfer RNA. The subnuclear site of the synthesis of messenger RNA in the animal cells is not clearly defined. Our experimental results show that: (1) the growth hormone stimulates the synthesis of rapidly-labelled RNA in the nucleus; (2) this labelled fraction passes to the cytoplasm and more appropriately to the microsomes on prolonged incubation; (3) actinomycin D inhibits its synthesis (Table 6); (4) the RNA stimulated by the hormone is a heterogeneous polydisperse fraction probably composed of more than one type of molecular species.

We are thus tempted to speculate that the hormone influences the synthesis of both the ribosomal RNA and messenger RNA, and that the nucleolar RNA consists of not only the ribosomal RNA but also the messenger RNA. Only in such circumstances would it fulfil the requirements of increased protein synthesis caused by the hormone action.

The hormone may induce the 'transcription' of hitherto repressed or dormant genetic information or may merely stimulate the existing activities. Allfrey, Littau & Mirsky (1963) have reported that 80 % of the genetic information is latent in animal cells in the interphase state. In the larvae of

Chironomus tentans, a morphogenetic hormone, ecdysone, causes a 'puffing' of a series of chromosomal segments, which appear as active sites of incorporation of labelled uracil on radioautography (Clever & Karlson, 1960; Pelling, 1959). Our results are insufficient to ascertain whether the growth hormone activates the synthesis of a new type (or types) of RNA or whether it modifies only the steady-state labelling of some existing RNA fractions, either by increasing the rate of synthesis or by diminishing the rate of breakdown. The apparent molar base ratios of the N-3 nucleolar fractions from hormone-treated rats differ from the normal pattern (Table 5). In a single homogeneous molecular species it would have indicated the emergence of a new type of RNA, but in a mixed population this could also be due to an increase in the proportion of one (or more) of the component types.

SUMMARY

1. Growth hormone stimulates the incorporation *in vivo* of [32 P]orthophosphate and [$6\text{-}^{14}\text{C}$]orotic acid into rat-liver RNA. The initial stimulation occurs in the nuclear fractions of RNA, but with longer incorporation times the microsomal fractions of RNA also become highly labelled.

2. The nuclear RNA has been fractionated into three fractions that have been analysed on a sucrose-density gradient. The administration of growth hormone increases particularly the specific radioactivity of the nucleolar RNA fraction. This stimulation is inhibited by actinomycin D.

3. The molar base ratios of these fractions in liver nuclei from normal and growth-hormone-treated rats have been determined. The hormone does not cause any qualitative change in the N-2 subfraction (RNA extracted with DNA-proteins in 1M-sodium chloride), but the base composition of the heterogeneous polydisperse nucleolar RNA is altered.

4. It is suggested that the hormone may primarily stimulate the synthesis of ribosomal RNA and messenger RNA.

We thank Dr B. B. Dikshit and Dr K. L. Wig for encouragement. A part of the work reported here was made possible by a travel grant to G. P. T. for which gratitude is expressed to Professor J. Monod and the Department of Cultural Relations. The work was supported by a research grant from the Atomic Energy Department. Shri G. S. K. Rao gave valuable technical assistance.

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Studies on the Structure of a Pigment Related to Chlorophyll *a* Produced by *Rhodopseudomonas spheroides*

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(Received 15 November 1963)

When *Rhodopseudomonas spheroides* is grown in a medium which contains 8-hydroxyquinoline, bacteriochlorophyll synthesis is partly inhibited and a number of pigments accumulate in the cells and medium (Jones, 1963b); these may be intermediates, or related to intermediates, in the biosynthesis of bacteriochlorophyll (Jones, 1963c). One of these compounds is magnesium 2,4-divinylphaeophorphyrin α_5 monomethyl ester (Jones, 1963c), and another is spectroscopically identical with phaeophorbid *a*, the magnesium-free derivative of chlorophyllide *a*.

One of the pigments (compound 2 of Jones, 1963b), which was isolated from the inhibited cells and medium, corresponds very closely in spectroscopic properties with the magnesium-free derivative of *Chlorobium* chlorophyll (650), the photosynthetic pigment of certain bacteria (Stanier & Smith, 1960). Compound 2 differs from *Chlorobium* phaeophorbid (650) in that it gives a positive reaction in the Molisch phase test for the presence

of an isocyclic ring substituted with a carboxylic group, a reaction given by chlorophyll *a*, bacteriochlorophyll and the magnesium-free derivatives of these compounds (phaeophytins).

In the present paper are described studies on the structure of compound 2 which have led to its identification as 2-devinyl-2-hydroxyethylphaeophorbid *a*. Its possible significance in the biosynthesis of bacteriochlorophyll is discussed.

MATERIALS AND METHODS

Hydrochloric acid solutions. To conform with the usual convention in this field concentrations of HCl are given as % (w/v).

Tetrapyrrole compounds. Phaeophorbid *a* was a gift from Dr J. E. Falk. 2-Devinyl-2-hydroxyethylmethylphaeophorbid *a* was a gift from Mr J. Barrett, Royal North Shore Hospital, Sydney, Australia. It was further purified by chromatography on a cellulose column (30 cm. $\times$ 2 cm.) equilibrated with light petroleum (b.p. 40–60°)–diethyl ether (10:1, v/v). The column was developed with light petroleum containing increasing concentrations of ether (cf. Morell, Barrett & Clezy, 1961). Protoporphyrin mono-

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