Effect of Growth Hormone on Ribonucleic Acid Metabolism

1. INCORPORATION OF RADIOACTIVE PHOSPHATE INTO RIBONUCLEIC ACID FRACTIONS OF RAT LIVER

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Di Stefano & Diermeier (1959) have observed a decrease in liver RNA and protein in hypophysectomized rats. Administration of growth hormone restored the levels of liver RNA and protein in these animals. Sesso & Valeri (1958) noted that growth hormone increased the DNA and RNA content of the atrophic pancreas in hypophysectomized rats and concluded that the primary effect of growth hormone is to increase cellular multiplication without modifying appreciably the cytoplasmic growth or the per-cell content of the nucleic acids. In this paper we have examined the effects of growth-hormone treatment and of partial hepatectomy on the incorporation of labelled orthophosphate into RNA from several subcellular fractions of rat liver.

MATERIALS AND METHODS

Animals. Six-month-old female (plateaued) albino rats (Malaria Institute strain) were used with litter-mates as controls. The rats were deprived of food overnight.

Growth hormone. Antuitrin-G (Parke, Davis and Co. Ltd.) was used and diluted in sterilized 0.9% NaCl, so that each millilitre contained 10 units. The biological potency of the material was confirmed by assay on female plateaued rats (Evans & Simpson, 1931). The weights of the treated rats increased by 16–26%, whereas those of the controls did not fluctuate beyond 5–7%.

Partial hepatectomy. One lobe of liver was removed under ether anaesthesia. After 26 hr. the rat was killed and the liver removed.

Radioactive phosphorus. ^{32}P as orthophosphate in sterile 0.9% NaCl (Atomic Energy Establishment, Trombay) was injected intraperitoneally (100 μC /100 g. of rat).

Removal of the tissue. The animals were killed by decapitation. The liver was perfused through the hepatic vein with cold 0.25M-sucrose; it was weighed and homogenized in a mixture of (a) 8 vol. of 0.25M-sucrose containing 3.3 mM- CaCl_2 and (b) 1 vol. of 0.5M-sucrose (final concentration: sucrose 0.28M, CaCl_2 2.9 mM). The homogenization was done in a Potter-Elvehjem (Potter, 1955) type of homogenizer fitted with a Teflon pestle.

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Preparation of subcellular fractions. Sucrose nuclei were separated according to Allfrey, Mirsky & Osawa (1957), and the nuclear soluble and nucleolar fractions were obtained according to Allfrey & Mirsky (1957). The supernatant after removal of nuclei served for the preparation of mitochondrial, microsomal and cytoplasmic soluble fractions according to the procedure described by Osawa, Takata & Hotta (1958). The mitochondrial fraction was discarded. Soluble RNA was prepared according to Schweet, Lamform & Allen (1958).

Extraction and determination of ribonucleic acid. RNA from the subcellular fractions was extracted and determined by the micromethod of Scott, Fraccastoro & Taft (1956). This method gave results identical with those obtained by the method of Kay & Dounce (1953) with sodium dodecyl sulphate for extraction.

Radioactivity measurements. Samples of the extracted RNA (100 μg .) were plated uniformly on a glass planchet which had been treated with a drop of Teepol 'L' (British Drug Houses Ltd.) to ensure uniform spreading. The sample was dried in a thin layer with an infrared lamp on a sample spinner (Nuclear Instruments, Chicago). The radioactivity was measured with a Geiger-Müller tube TGC-2 (window thickness, 1.8 mg./cm.²). Measurements were made in general for 10–15 min. (keeping time constant) and for a minimum of 1000 counts (background, 25 counts/min.). Counts were corrected to the time of injection by using decay curves. No correction for self-absorption was considered necessary.

Estimation of phosphorus. The total fraction was digested in conc. H_2SO_4 and phosphorus was estimated by Sumner's (1944) method.

Calculation of specific activity. Specific activity was calculated as counts/min./ μg . of P. Relative specific activity was calculated as counts/min./ μg . of P/ μC of ^{32}P dose injected/g. body wt.

RESULTS

Effect of growth hormone or partial hepatectomy on ^{32}P incorporation in different fractions of liver ribonucleic acid

Antuitrin-G was injected intraperitoneally (6 units/100 g. body wt.) 6 hr. before the removal of tissues for RNA estimations. A similar procedure was used by Knobil, Franklin & Hotchkiss (1960) in studying the effect of growth hormone on fatty acid oxidation. Five hours after the administration

Table 1. *Specific activity of ^{32}P in ribonucleic acid from subcellular fractions of livers from normal, partially hepatectomized and growth-hormone-treated rats*

Growth hormone (6 units/100 g. rat body wt.) was injected intraperitoneally 5 hr. before $\text{Na}_2\text{H}^{32}\text{PO}_4$ (100 μC /100 g. body wt.). Animals were killed and tissues removed 1 hr. later. Experimental details were as indicated in the text. Partial hepatectomy was done 26 hr. before removal of tissues, and the isotope in the same dosage was injected 1 hr. before the animal was killed. Figures give mean values of relative specific activity $\pm$ S.E.M. with numbers of experiments and ranges in parentheses.

	Normal	Growth-hormone treated	Change	Regenerating	Change
Nuclear soluble RNA	41.1 $\pm$ 6.9 (6; 25.3-65.7)	65.7 $\pm$ 12.0 (5; 41.5-105.3)	+59.9% $P < 0.1$	95.1 $\pm$ 17.2 (5; 58.7-146.2)	+131.4% $P < 0.02$
Nucleolar RNA	97.8 $\pm$ 27.5 (5; 29.9-185.6)	274.4 $\pm$ 69.2 (4; 149.1-472.1)	+180.6% $P < 0.05$	269.1 $\pm$ 44.1 (5; 171.6-382.7)	+175.2% $P < 0.01$
Microsomal RNA	136.1 $\pm$ 27.9 (5; 60.9-215.2)	350.5 $\pm$ 67.8 (5; 157.1-511.9)	+157.5% $P < 0.02$	330.8 $\pm$ 71.5 (5; 131.9-546.4)	+143.1% $P < 0.05$
Cytoplasmic soluble RNA	51.4 $\pm$ 12.3 (6; 14.8-99.9)	94.0 $\pm$ 14.9 (4; 67.6-126.1)	+82.9% $P > 0.05$	141.0 $\pm$ 13.9 (5; 93.6-179.0)	+174.3% $P < 0.001$

Table 2. *Ribonucleic acid content of subcellular fractions from livers of normal, growth-hormone-treated and partially hepatectomized rats*

Growth hormone (6 units/100 g. rat body wt.) was injected intraperitoneally 6 hr. before removal of tissue. Partial hepatectomy was done 26 hr. before the animals were killed. Experimental details were as indicated in the text. Figures express mean value of RNA content ($\mu\text{g.}/\text{g.}$ of liver) $\pm$ S.E.M. with numbers of experiments and ranges in parentheses.

	Normal	Growth-hormone treated	Change	Regenerating	Change
Nuclear soluble RNA	490.2 $\pm$ 39.0 (5; 412.0-623.9)	418.5 $\pm$ 55.8 (3; 338.9-526.3)	-14.6% $P > 0.3$	731.9 $\pm$ 48.7 (3; 637.8-792.0)	+49.3% $P < 0.01$
Nucleolar RNA	644.1 $\pm$ 54.0 (5; 528.2-829.9)	1374.6 $\pm$ 232.9 (3; 1089.5-1835.6)	+113.4% $P < 0.02$	2051.3 $\pm$ 347.3 (3; 1514.2-2701.6)	+218.5% $P < 0.01$
Microsomal RNA	2254.7 $\pm$ 264.3 (5; 1739.3-2916.1)	1409.9 $\pm$ 66.8 (3; 1276.8-1483.7)	-37.5% $P = 0.02$	2318.1 $\pm$ 169.4 (3; 2074.3-2643.8)	+2.8% $P = 0.8$
Cytoplasmic	670.1 $\pm$ 78.5 (5; 455.4-845.0)	591.1 $\pm$ 123.2 (3; 430.3-832.8)	-11.8% $P = 0.6$	833.5 $\pm$ 89.4 (3; 658.9-945.8)	+24.4% $P > 0.2$

of growth-hormone preparation, $\text{Na}_2\text{HP}^{32}\text{O}_4$ (100 μC /100 g. body wt.) was injected intraperitoneally and the animal killed by decapitation 1 hr. after the isotope dose. The liver was perfused with cold isotonic sucrose (0.25M) and various RNA fractions were extracted as described under Materials and Methods. Table 1 summarizes the results. Growth-hormone injection increased the specific activity of ^{32}P in all fractions of RNA and particularly in the nucleolar and microsomal fractions. In regenerating liver the increase was almost the same in all the four fractions (Table 1). Table 2 shows that growth hormone caused a net increase in the quantity of RNA/g. of liver only in the nucleolar RNA fraction. The nuclear soluble and cytoplasmic soluble RNA did not undergo any significant variation, but the amount of microsomal RNA fell. The specific activity of ^{32}P in microsomal RNA, however, increases on growth-hormone administration. In the regenerating tissues there is a net increase in the RNA content of the nuclear soluble

and nucleolar RNA fractions (Table 2). The change in microsomal RNA is not significant. The cytoplasmic soluble RNA is increased by 24.4%.

DISCUSSION

The results suggest that growth hormone acts principally on the particulate fractions, viz. nucleolus and microsomes. There is an increased incorporation of ^{32}P , as well as an increase in the total quantity of RNA in the nucleolar fraction per unit weight of tissue. Sirlin (1960) and Fitzgerald & Vinijchaikul (1959) have suggested the nucleolus as the seat of RNA synthesis. Other authors (Woods & Taylor, 1959; Goldstein & Micou, 1959) believe that the initial biosynthesis of RNA takes place around the chromatin material which subsequently passes through the nucleolus. The existence of a dual mechanism, i.e. synthesis around chromatin material and in nucleolus independently, has also been hinted (Gall, 1959; Amano & Leblond,

1960). In view of the uncertain interrelationship between the nucleolus and nuclear compartment, it is difficult to gauge whether the growth hormone effect is directly on the nucleolus or a consequence of a primary effect on some other sequence in the nucleus.

Growth hormone might also modify the transfer rate of nuclear RNA into cytoplasmic compartments. Work of several authors has indicated a rapid transfer of RNA from the nucleus to the cytoplasm (Goldstein & Plaut, 1955; Zalokar, 1960; Amano & Leblond, 1960; Perry, 1960; Goldstein & Micou, 1959; Pelling, 1959). A decrease in the quantity of cytoplasmic fractions, particularly the microsomal, on growth-hormone treatment with a simultaneous increase of specific activity of this fraction would imply that the consumption or catabolic turnover of microsomal RNA is outstripping the replenishment rate (whether from synthesis *in situ* or that derived from the nucleus). These effects may conceivably be connected with the increased protein synthesis that occurs on administration of growth hormone (Russell, 1955; Korner, 1959, 1960).

In the regenerating tissues, the increase in the quantity of RNA in nuclear fractions is significantly higher than the change in the cytoplasmic fractions.

SUMMARY

1. The effects of growth-hormone treatment and of partial hepatectomy on the incorporation of labelled orthophosphate into ribonucleic acid from several subcellular fractions (nuclear soluble, nucleolar, cytoplasmic soluble and microsomal) of rat liver have been studied.

2. The specific activity of ^{32}P increased in all fractions of liver ribonucleic acid on growth-hormone treatment, but the increase in nucleolar and microsomal fractions is significantly marked.

3. In regenerating liver there was a uniform increase in specific activity of ^{32}P in all fractions.

4. On growth-hormone administration, a quantitative increase in the content of ribonucleic acid over the normal levels occurs only in the nucleolar fraction, the content of microsomal ribonucleic acid showing a decline.

5. In regenerating liver, the major quantitative

increase is in the nuclear fraction (nuclear soluble 49.3 %, nucleolar 218.5 %).

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