Effect of Growth Hormone on Ribonucleic Acid Metabolism

2. EFFECT OF GROWTH-HORMONE ADMINISTRATION ON THE ACTIVITIES OF 5'-NUCLEOTIDASE, ADENASE, GUANASE AND XANTHINE OXIDASE

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Talwar, Panda, Sarin & Tolani (1961) have found that growth hormone causes an increase in the specific activity of ^{32}P in RNA of cytoplasmic fractions, whereas the total RNA content of these fractions diminishes in comparison with normal levels. Stevens & Reid (1956) have reported that hypophysectomy or adrenalectomy causes an increase in the activities of acid deoxyribonuclease and acid ribonuclease in rat-liver cytoplasm. No increase was noticed in the activity of the ribonuclease with optimum pH of 8. The same authors did not notice any change in acid-ribonuclease activity in the liver mitochondrial and microsomal fractions of the hypophysectomized rats. They, however, observed an increase in the activity of this enzyme in the particulate fractions on treatment with growth hormone.

Reid, O'Neal & Lewin (1956) have further noticed that hypophysectomy led to an increase in xanthine-oxidase activity, whereas adrenalectomy decreased this activity. This effect of hypophysectomy was not altered by administration of growth hormone. Reid & Stevens (1958) have further seen that nucleotidase activity decreases by 30% in hypophysectomized rats. No significant change was noticed in the activities of uricase, adenosine deaminase and purine nucleoside phosphorylase.

In this paper activities of 5'-nucleotidase, adenase, guanase and xanthine oxidase have been assayed in livers from normal and growth-hormone-treated rats.

MATERIALS AND METHODS

Animals. Albino rats (Malaria Institute strain) of different age groups were used.

Growth hormone. Antuitrin-G (from Parke, Davis and Co. Ltd.) was used dissolved in sterilized 0.9% NaCl. Growth-hormone preparation (0.5–1 unit) was injected daily intraperitoneally for a period of 2–3 weeks before the animal was killed. The biological activity of the growth-hormone sample was confirmed as described earlier (Talwar *et al.* 1961).

Removal of tissue for enzyme assays. The animals were killed by decapitation. Liver was removed quickly and chilled in water. Approximately 1 g. of liver was taken and homogenized in 10 ml. of ice-cold water in a Potter-Elvehjem (Potter, 1955) type of homogenizer. A measured sample of the homogenate was taken for DNA estimation. The rest was centrifuged in an International refrigerated centrifuge at 3° at 2000 rev./min. for 10 min. The supernatant obtained retains the activity of enzymes reported here (Lamirande, Allard & Cantero, 1958). A known volume was diluted ten times and was used for the enzyme assays.

Estimation of nitrogen. The N content of the homogenates containing enzymes was determined by the micro-Kjeldahl method (Grabar, 1954).

Estimation of protein. The protein content of the homogenate containing the enzyme was determined by the spectrophotometric method of Warburg & Christian (1941).

Estimation of deoxyribonucleic acid. DNA was determined by the micromethod of Scott, Fraccastoro & Taft (1956).

Preparation of substrates. Solutions (0.02M) of adenine, guanine and xanthine (California Foundation for Biochemical Research) were prepared in 0.025N-NaOH with gentle heating. For the experiments in Table 4, 1 mM-guanine in 0.01N-NaOH was employed.

A solution (5.3 mM) of AMP (Fluka A.G., Chemische Fabrik, Buchs, Switzerland) solution was prepared in water.

Determination of enzyme activities

5'-Nucleotidase. Activity was determined by the method of Heppel & Hilmoe (1955) except that the AMP concentration taken for estimation was 4 μmoles (in 1.7 ml. of incubation mixture). A total volume of incubation mixture of 1.7 ml. contained 0.75 ml. of 5.3 mM-AMP, 0.1 ml. of 0.1M-MgCl₂, 0.1 ml. of M-glycine buffer, pH 8.5, 0.25 ml. of water and 0.5 ml. of approximately 10% homogenized centrifuged tissue suspension. After 15 min. at 37°, the reaction was stopped with 1.7 ml. of 10% trichloroacetic acid. Inorganic phosphorus liberated was determined in duplicate by the method of Sumner (1944).

Adenase, guanase and xanthine oxidase. These enzymes were estimated by a procedure similar to that described by Schneider & Hogeboom (1952) for uricase and by Kalckar (1947) for estimation of purines. A total volume of incubation mixture of 6.4 ml. contained 0.2 ml. of 0.02M-solution of respective substrates (at this concentration these substrates are nearly at their maximum solubility limit), 0.5 ml. of 0.5M-potassium phosphate buffer, pH 7.4, 4.7 ml. of water and 1 ml. of approximately 1% centrifuged homogenate. Duplicate mixtures were used for each assay. After 15 min. at 37°, the reaction was stopped with

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Table 1. *Effect of growth hormone on enzyme activities in liver of 3-week-old rats*

Antuitrin-G (0.5 unit) was injected intraperitoneally every day for 3 weeks. Figures give mean values of enzyme units/mg. of nitrogen or /100 μ g. of DNA. An enzyme unit of guanase and xanthine oxidase is the amount of enzyme producing a change of 0.1 in extinction of the respective substrate at the wavelengths indicated in the text, in 15 min. at 37°. A unit of nucleotidase is the enzyme liberating 1 μ g. of P in 15 min. under the conditions of the assay. Standard errors of the mean have been indicated with numbers of experiments and ranges of observed values in parentheses. No significant change was found in adenase activity, which was present in traces only. 'Traces' indicate activities < 0.1 unit.

	Enzyme units/mg. of N			Enzyme units/100 μ g. of DNA		
	Normal	Growth-hormone treated	Change	Normal	Growth-hormone treated	Change
5'-Nucleotidase	25.4 $\pm$ 7.2 (6; 12.1-59.5)	77.6 $\pm$ 1.1 (4; 75.0-80.3)	+205.5% <i>P</i> < 0.001	37.8 $\pm$ 3.6 (6; 26.9-50.3)	79.1 $\pm$ 7.3 (4; 61.3-96.9)	+109.2% <i>P</i> < 0.001
Guanase	4.5 $\pm$ 1.3 (6; 1.2-8.4)	15.1 $\pm$ 0.3 (4; 14.4-15.9)	+235.5% <i>P</i> < 0.001	6.5 $\pm$ 1.2 (6; 2.8-10.2)	15.2 $\pm$ 1.3 (4; 12.1-18.4)	+133.8% <i>P</i> < 0.001
Xanthine oxidase	2.0 $\pm$ 0.7 (6; 0.1-4.3)	0.7 $\pm$ 0.8 (4; Traces-1.5)	-65.0% <i>P</i> < 0.1	3.0 $\pm$ 1.02 (6; 0.4-8.2)	0.9 $\pm$ 0.3 (4; Traces-1.9)	-70.0% <i>P</i> < 0.1

Table 2. *Effect of growth hormone on enzyme activities in liver of 2-month- and 6-month-old rats*

Antuitrin-G (1 unit) was injected intraperitoneally daily for 2 weeks. Figures express mean values of enzyme activities in units/100 μ g. of DNA $\pm$ s.e.m. with numbers of experiments and ranges in parentheses. No significant change was observed in the activity of adenase.

	2-Month-old			6-Month-old		
	Normal	Growth-hormone treated	Change	Normal	Growth-hormone treated	Change
5'-Nucleotidase	45.5 $\pm$ 1.4 (3; 43.1-47.9)	54.3 $\pm$ 3.1 (4; 46.6-62.0)	+19.3% <i>P</i> < 0.05	39.1 $\pm$ 2.8 (4; 34.3-45.9)	41.2 $\pm$ 2.3 (4; 35.5-46.9)	+5.3% <i>P</i> = 0.6
Guanase	14.2 $\pm$ 0.6 (3; 13.2-15.3)	18.6 $\pm$ 2.7 (4; 12.0-25.3)	+31.0% <i>P</i> = 0.2	6.9 $\pm$ 2.7 (3; 3.9-9.7)	27.7 $\pm$ 0.2 (4; 27.3-28.2)	+301.4% <i>P</i> < 0.001
Xanthine oxidase	Traces	Traces	—	2.7 $\pm$ 1.05 (4; Traces-5.2)	0.3 $\pm$ 0.15 (4; Traces-0.7)	-88.8% <i>P</i> > 0.05 < 0.1

2 ml. of 10% trichloroacetic acid. In the zero-minute reading trichloroacetic acid was added to the incubation mixture before the addition of the enzyme. The extinction coefficients of the supernatants after centrifuging were measured in a Hilger Uvispek spectrophotometer at the following wavelengths: adenine, 259 $m\mu$; guanine, 245 $m\mu$; xanthine, 273 $m\mu$. These wavelengths correspond to the maximum absorption of the respective purines at the pH of measurement. The extinction change serves as an index of the substrate depletion.

For the experiments of Table 4, guanase activity was measured with 0.3 ml. of 0.1M-glycine-NaOH buffer, pH 9, 0.6 ml. of 1 mM-guanine solution, and 0.1 ml. of approximately 10% centrifuged homogenate. The mixture was incubated for 5 min. at 37°, after which the reaction was stopped by addition of 1 ml. of 10% HClO₄ and final volume was made up to 3 ml. with 1 ml. of water. The change in extinction of the supernatant was observed at 248 $m\mu$ and at 282 $m\mu$. The former wavelength corresponds to the absorption maxima of guanine at the pH of measurement, whereas at 282 $m\mu$ the absorption due to xanthine in the medium is minimized. Double-distilled water was used for all these solutions.

Units of enzyme activity. A unit of adenase, guanase or xanthine oxidase is the amount of enzyme that produces a change of 0.1 in extinction of the respective substrate ($\Delta E_{1\text{cm.}}^{\lambda\text{max.}}$) in 15 min. at 37°.

A unit of 5'-nucleotidase is the amount of enzyme that liberates 1 μ g. of phosphorus in 15 min. under the conditions of the assay.

Specific activity of guanase. In Table 4, $\Delta E_{1\text{cm.}}^{248}$ or $\Delta E_{1\text{cm.}}^{282}$ represents the change in extinction at 248 $m\mu$ or at 282 $m\mu$ respectively in 5 min. of enzyme action/mg. of protein.

RESULTS

Antuitrin-G (0.5 unit) was injected intraperitoneally every day for 3 weeks into the first group of rats and 1 unit daily for 2 weeks was the dose injected into rats in groups 2 and 3. Litter mates were kept as controls in each group. The enzyme activities in liver of normal and of growth-hormone-injected rats in group 1 are presented in Table 1. An appreciable increase occurs in 5'-nucleotidase and guanase activities, whereas the xanthine-oxidase activity decreases in growth-hormone-injected rats.

The results obtained with 2-month-old actively growing rats are given in Table 2. The changes in enzyme activities in this group are less marked than in group 1. Table 2 also summarizes the results obtained with 6-month-old plateaued rats. The

Table 3. *Biological effect of growth hormone on rats of different ages*

Growth hormone (0.5 units) was injected intraperitoneally daily into 3-week-old rats for 3 weeks and 1 unit daily for 2 weeks was the dose for 2-month-old and 6-month-old rats. Body weights were recorded every day. Litter mates were kept as controls. Mean values of body weight in g. are given $\pm$ s.e.m. for four animals in each group.

Initial age of rats	Control group			Growth-hormone treated		
	Initial	Final	Change (%)	Initial	Final	Change (%)
3 weeks	29.0 $\pm$ 0.9	72.2 $\pm$ 0.8	149.6 $\pm$ 5.4 ($P < 0.001$)	26.7 $\pm$ 1.8	77.5 $\pm$ 3.2	191.4 $\pm$ 11.2 ($P < 0.001$)
2 months	79.7 $\pm$ 1.5	116.0 $\pm$ 2.1	45.4 $\pm$ 0.9 ($P < 0.001$)	77.5 $\pm$ 2.0	115.0 $\pm$ 4.4	48.8 $\pm$ 7.7 ($P < 0.01$)
6 months	126.0 $\pm$ 1.0	131.5 $\pm$ 2.1	4.3 $\pm$ 1.5 ($P > 0.05 < 0.1$)	117.7 $\pm$ 6.1	142.2 $\pm$ 8.7	20.7 $\pm$ 2.4 ($P < 0.01$)

Table 4. *Effect of growth hormone on specific activity of guanase and 5'-nucleotidase in rat liver*

Growth hormone (6 units/100 g. body weight) was injected intraperitoneally into plateaued female rats 6 hr. before removal of tissues. $\Delta E_{1\text{cm}}^{248}$ or $\Delta E_{1\text{cm}}^{282}$ represents change in extinction at 248 m μ or 282 m μ /5 min./mg. of protein. The reaction was stopped by addition of perchloric acid. Extinctions were determined with the supernatant. Nucleotidase activity is expressed as μ g. of P liberated/15 min./mg. of protein. Results are given as means with ranges in parentheses for three experiments.

		Control	Growth-hormone treated	Change
Guanase	$\Delta E_{1\text{cm}}^{248}$	27.4 $\pm$ 4.5 (19.2-34.9)	35.6 $\pm$ 2.6 (32.5-40.8)	+29.9% $P > 0.2$
	$\Delta E_{1\text{cm}}^{282}$	49.7 $\pm$ 5.7 (38.4-55.6)	61.7 $\pm$ 2.2 (57.6-65.1)	+24.2% $P > 0.1$
5'-Nucleotidase		1.8 $\pm$ 0.2 (1.4-2.2)	1.6 $\pm$ 0.2 (1.3-2.0)	-11.1% $P > 0.1$

principal increase occurs in guanase activity. In all groups similar changes are recorded, though their magnitude is minimal for 2-month-old actively growing rats. Increase in body weight is the basis of the procedure of Evans & Simpson (1931) for estimation of growth-hormone potency. Exogenous growth-hormone administration causes the least increase of body weight over normal values in 2-month-old actively growing rats (Table 3). In the same group the enzyme changes are also minimal.

The experiments reported above have been conducted on animals where growth-hormone administration was continued over a period of 2-3 weeks. In the preceding paper (Talwar *et al.* 1961) growth-hormone effect on biosynthesis of RNA was studied 6 hr. after administration of a dose of growth hormone (6 units/100 g. body wt.). Activities of 5'-nucleotidase and guanase were estimated in acute experiments with 6-month-old female plateaued rats (Table 4). Though hardly any change is observable in 5'-nucleotidase activity there occurs about 25% increase in the specific activity of guanase in growth-hormone-treated rats.

DISCUSSION

Talwar *et al.* (1961) found an increased turnover of cytoplasmic RNA fractions in growth-hormone-treated plateaued rats. Reid & Stevens (1958) observed an increase of acid-ribonuclease activity in hypophysectomized growth-hormone-treated rats. In this paper an increase in guanase and a decrease in xanthine oxidase have been noted.

The effect on guanase activity occurs only in those age groups where growth-hormone administration is also able to cause an increase in the body weight of the animal (namely 3-week-old rats and 6-month-old female rats). No effect was noticed on 2-month-old rats. It is also observed that in 3-week-old rats, 5'-nucleotidase activity is increased along with the increase in guanase.

Hypophysectomy causes a fall in the ability of the liver to incorporate amino acids into proteins *in vivo* (Korner, 1960) or in a cell-free system (Korner, 1959). Treatment of hypophysectomized rats with growth hormone restored the ability to synthesize proteins at the rate found in normal rat liver. Korner's observations suggest that the lesion lies principally in the transfer process between

soluble RNA-amino acid complex and microsomes and only to a lesser degree in the steps involving amino acids activation and transport. This step requires GTP as a cofactor. It is thus conceivable that an increased synthesis of proteins may be accompanied by an increased turnover of guanine nucleotides. Increase in 5'-nucleotidase and guanase activity of the cells as brought about by growth-hormone administration may be connected with an increased turnover of guanine nucleotides.

Growth-hormone administration is also seen to cause a decrease in xanthine-oxidase activity. Bergel, Bray, Haddow & Lewin (1957) have ascribed a key role to this enzyme in the purine cycle. Its activity is seen to decrease in phases of accelerated growth (Lamirande *et al.* 1958).

SUMMARY

1. Activities of 5'-nucleotidase, adenase, guanase and xanthine oxidase have been estimated in livers from normal and growth-hormone-treated rats.

2. Increased 5'-nucleotidase and guanase activities were found after growth-hormone administration; xanthine-oxidase activity fell.

Part of the results reported here are included in the M.Sc. thesis of N. C. Panda. Gift of Antuitrin-G from Parke, Davis and Co. Ltd. is gratefully acknowledged.

REFERENCES

- Bergel, F., Bray, R. C., Haddow, A. & Lewin, I. (1957). *Ciba Foundation Symp., The Chemistry and Biology of Purines*, pp. 256-69.
- Evans, H. M. & Simpson, M. E. (1931). *Amer. J. Physiol.* **98**, 511.
- Grabar, P. (1954). In *Techniques de Laboratoire*, p. 828. Ed. by Loiseleur, J. Paris: Masson et Cie.
- Heppel, L. A. & Hilmo, R. J. (1955). In *Methods in Enzymology*, vol. 2, p. 546. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press, Inc.
- Kalckar, H. M. (1947). *J. biol. Chem.* **167**, 429, 445, 461.
- Korner, A. (1959). *Biochem. J.* **73**, 61.
- Korner, A. (1960). *Biochem. J.* **74**, 462.
- Lamirande, G., Allard, C. & Cantero, A. (1958). *Cancer Res.* **18**, 952.
- Potter, V. R. (1955). In *Methods in Enzymology*, vol. 1, p. 10. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
- Reid, E., O'Neal, M. A. & Lewin, I. (1956). *Biochem. J.* **64**, 730.
- Reid, E. & Stevens, B. M. (1958). *Biochem. J.* **68**, 367.
- Schneider, W. C. & Hogeboom, G. H. (1952). *J. biol. Chem.* **195**, 161.
- Scott, J. F., Fraccastoro, A. P. & Taft, E. B. (1956). *J. Histochem. Cytochem.* **4**, 1.
- Stevens, B. M. & Reid, E. (1956). *Biochem. J.* **64**, 735.
- Sumner, J. B. (1944). *Science*, **100**, 413.
- Talwar, G. P., Panda, N. C., Sarin, G. S. & Tolani, A. J. (1961). *Biochem. J.* **82**, 173.
- Warburg, O. & Christian, W. (1941). *Biochem. Z.* **310**, 384.

Biochem. J. (1962) **82**, 179

Intracellular Distribution and Biosynthesis of Ubiquinone in Rat Liver in Carbon Tetrachloride Liver Injury

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The fundamental metabolic deviation by which carbon tetrachloride administration results in fatty degeneration of liver is not yet clear. In recent reports on the changes in composition and function of the fatty liver cell particular attention has been paid to the mitochondria. A damage to mitochondrial integrity is accompanied by major biochemical alterations (Dianzani, 1954, 1955, 1956; Dianzani & Viti, 1955; Kasbekar, Lavate, Rege & Sreenivasan, 1959). Inhibition of certain enzymes (Dianzani, 1953) and activation of others (Lehninger, 1951; Berthet & de Duve, 1951; Schneider & Hogeboom, 1952*a, b*; Hogeboom & Schneider, 1953) latent in fresh mitochondria, especially of adenosine triphosphatase (Kielley & Kielley, 1951;

Potter & Recknagel, 1951; Copenhaver & Lardy, 1952; Lardy & Wellman, 1952, 1953) have been reported.

Dianzani & Viti (1955) have observed decreases in both cytochrome *c* and pyridine nucleotides in fatty livers. Work in this Laboratory (Nadkarni, Wagle & Sreenivasan, 1957) has further shown that the biosynthesis *in vivo* of pyridine nucleotides from intraperitoneally administered nicotinamide is less in the carbon tetrachloride-treated animals. A decrease in hepatic coenzyme A has also been reported in fatty degeneration (Severi & Fonnesu, 1956; Heim, Leuschner & Ott, 1956). In view of the changes in cytochrome *c* and pyridine nucleotides, and in coenzyme A, which is known to be