INCREASED RIBONUCLEASE ACTIVITY OF NUCLEI OF LIVER CELLS OF THIOACETAMIDE-TREATED RATS*

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Received September 29, 1964

INTRODUCTION

The increased content of nucleolar RNA produced by thioacetamide has been shown to be associated with an increased overall uptake of labeled precursors into nucleolar RNA *in vivo* (Adams and Busch, 1962; Steele *et al*, 1964). Concomitant with the increased content and biosynthesis of RNA in the nucleoli, there is a decreased content of RNA in the cytoplasm. Since nucleolar RNA is generally accepted as the precursor of ribosomal RNA (Busch *et al*, 1963; Scherrer *et al*, 1963; Birnsteil *et al*, 1964; Perry, 1964) it seemed that the decrease in cytoplasmic RNA could have resulted from a decreased activity of ribonucleases that cleave high molecular weight RNA to ribosomal RNA and/or an excessive activity of nuclear ribonucleases that hydrolyze the RNA as it leaves the nucleolus or the nuclear ribonucleoprotein network (Smetana *et al*, 1963). The present studies show that there is an 8-fold increase in ribonuclease activity in liver nuclei within 48 hours after administration of thioacetamide to rats.

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*These studies were supported in part by grants from the National Science Foundation, The American Cancer Society, the Jane Coffin Childs Fund and the U. S. Public Health Service CA 05421 and CA 07201.

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MATERIALS AND METHODS

Administration of Thioacetamide - Thioacetamide was injected intraperitoneally in a dose of 50 mg/kg body weight per day (Adams and Busch, 1963) into male albino rats (Holtzman Co.) weighing approximately 180-250 gm. The rats were sacrificed at 6, 12, 24 or 48 hours after initial injection of thioacetamide. Control rats were injected intraperitoneally with normal saline solution and were sacrificed simultaneously. Nuclei were isolated with minor modifications of the procedure of Chauveau et al (1956). The supernatant fraction containing the cytoplasm was diluted with sufficient cold distilled water to make the sucrose concentration 0.01 M.

Ribonuclease Assay - For assays of nuclear ribonuclease, each incubation vessel contained nuclei from 1 gm. of liver; for studies on cytoplasmic ribonuclease an aliquot of 1 ml. of the diluted solution was used. The nuclear fractions were suspended in 1 ml. of cold distilled water. The following were added to each incubation tube: 1 mg. ribosomal RNA, 0.8 ml. of buffer, and 1 mg. of bovine serum albumin (Kalnitsky et al, 1959). To each solution, 0.8 ml. of a 0.1 M acetate buffer was added for studies at pH 5; 0.8 ml. of 0.1 M Tris-HCl buffer was added for studies at pH 8.2. The final incubation volume was 2 ml. The contents of each reaction tube were thoroughly mixed using a Vortex Jr. mixer and then incubated for 30 minutes at 37°C. The incubation mixture was rapidly cooled in an ice bath. One ml. of 2.1 N perchloric acid was added to each tube. The supernatant fraction was centrifuged at 2,000 x g for 5 minutes or until it was clear. Changes in absorbancy were measured at 260 mμ; ribonuclease-free blanks were employed. The samples designated as zero time of incubation were kept

in ice for 30 minutes after addition of all components of the reaction mixture.

RESULTS

Nuclear Ribonuclease - Figures 1 and 2 show that the kinetics of hydrolysis of RNA were essentially linear in the systems employed over a 30 minute period. In the samples containing nuclei from livers of rats treated with thioacetamide for periods of 24 and 48 hours, the enzyme activities were increased 7 to 8-fold over those of nuclei of livers of

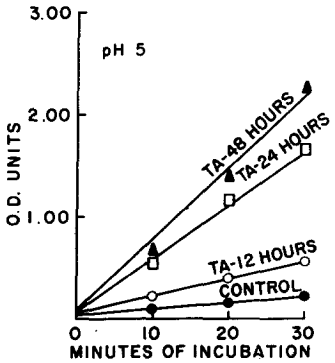

Figure 1. Kinetics of the hydrolysis of RNA by ribonuclease of isolated nuclei derived from 1 gm liver of thioacetamide-treated and control rats. Enzyme activity at pH 5 was measured by changes in absorbancy at 260 mμ.

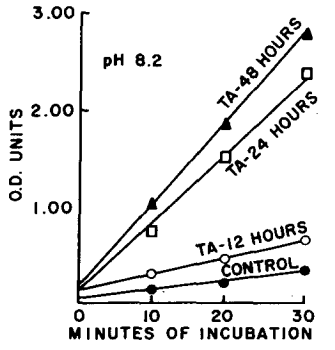

Figure 2. Same as Figure 1 with the exception that enzyme activity was determined at pH 8.2.

control rats. As also shown in Figure 3, the greatest increment in ribonuclease activity occurred in the period of 12 - 24 hours after the initial injection of thioacetamide. The increase in enzyme activity was essentially the same whether it was determined at pH 5 or pH 8.2. Although it was not marked, there was an increase in ribonuclease activity at 6 hours after the initial treatment with thioacetamide (Figure 3).

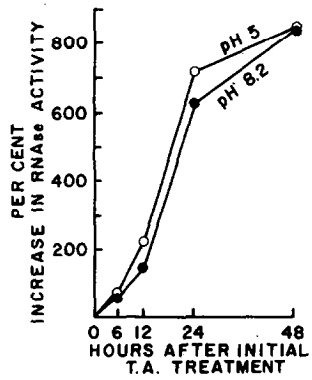

Figure 3. Increment in ribonuclease activity expressed in per cent of the control. Nuclei from liver cells were isolated at 6, 12, 24 or 48 hours after the initial administration of thioacetamide in a dose of 50 mg/kg/day to male rats. Enzyme activity was measured as indicated in Figures 1 and 2.

The increase in activity of the nuclear ribonuclease was not correlated with either an increase in the number of nuclei or of their diameters (Table I). In these preparations, the increase in nuclear size was inversely correlated with the number of nuclei in the preparation.

Cytoplasmic Ribonuclease - Over a period of 9 days following daily administration of thioacetamide to rats, there was no significant change in the activity of cytoplasmic ribonuclease. All values at time periods of ½, 1, 3, 6,

TABLE I

Effect of Thioacetamide on Diameters and Numbers of Nuclei of Liver Cells

The times of treatment are those after the initial administration of thioacetamide in a dose of 50 mg/kg. The nuclear diameters were measured directly in stained smears of nuclear preparations obtained by the method of Chauveau et al (1956). The results are averages of 100 determinations and the ranges are shown in parenthesis. The number of nuclei are averages of two experiments.

Treatment	Nuclear Diameter in μ	Number of Nuclei per gm. liver
Control	5.7 (5 - 7.5)	3.25×10^7
6 hours	5.98 (5 - 7.5)	3.15×10^7
12 hours	6.25 (5 - 7.5)	3.10×10^7
24 hours	6.84 (5.5 - 8.5)	2.90×10^7
48 hours	7.86 (5.5 - 9.0)	2.75×10^7

12, 24, and 48 hours as well as those at 4 and 9 days after initiation of thioacetamide administration were within the normal range.

Ribonuclease in regenerating liver nuclei - Inasmuch as enlargement of nucleoli has been noted in regenerating liver, these nuclei were also studied for their ribonuclease activity. Over a period of 6 and 18 hours following hepatectomy, there was no change in the activity of nuclear ribonuclease in these livers.

DISCUSSION

Recent studies have suggested that the remarkable increase in nucleolar size following administration of thioacetamide is related to an increased RNA synthesis in the nucleoli of liver cells (Steele et al, 1964; Villalobos et al, 1964). The concomitant decrease in the content and net synthesis

of cytoplasmic ribosomes (Adams and Busch, 1963) suggested that thioacetamide blocked the transport mechanisms involved in the release of nuclear precursors of ribosomes or that there was excessive destruction of the precursors of cytoplasmic ribosomes. The marked increase in nuclear ribonuclease activity found at early times after thioacetamide treatment suggests the possibility that increased nuclear breakdown of RNA is responsible for the destruction of precursors of cytoplasmic ribonucleoproteins. It is possible that the acceleration of nucleolar RNA synthesis reflects a loss of feedback controls.

The mechanism of enhancement of nuclear ribonuclease activity by thioacetamide is not yet clear since neither the possibility of stabilization of active enzyme, activation of latent enzyme, nor induction of new enzyme can be eliminated. What seems particularly interesting is that only the nuclear enzyme activity was increased (Harris, 1963; Hymer and Kuff, 1964). It is possible that this enzyme may be localized to the nucleus as is DPN-pyrophosphorylase (Schneider and Hogeboom, 1956).

SUMMARY

An eight-fold increase in nuclear ribonuclease activity was found 24 to 48 hours after administration of thioacetamide in a dose of 50 mg/kg. This effect was not associated with an increased activity of cytoplasmic ribonuclease, or increased numbers of nuclei. Moreover, nuclei of regenerating livers did not have an increased ribonuclease activity. The possibility exists that nuclear ribonucleases hydrolyze nuclear precursors of ribosomes in thioacetamide-treated animals and the increased nucleolar synthesis of RNA reflects

a loss of feedback controls.

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