

THE ROLE OF THE NUCLEOLUS

I. Tritiated Cytidine Activity in Liver Parenchymal Cells of Thioacetamide-Treated Rats

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ABSTRACT

Male rats of the Sherman strain were fed for 2 weeks a diet of ground purina rat chow containing 0.04 per cent thioacetamide. Animals were injected intraperitoneally with tritiated cytidine, $200\mu\text{c}/100\text{ gm}$ body weight, and sacrificed in pairs, a control and a thioacetamide-treated rat, at prescribed intervals. Liver tissues were preserved with the freeze-substitution method and postfixed in anhydrous OsO_4 . Other samples were fixed directly with an acetic acid-ethanol mixture (1:3). AR-10 stripping film was applied to 2- and 4- μ sections and exposed for appropriate lengths of time. Nuclear and nucleolar volumes were obtained by direct measurement. Cytoplasmic volumes were obtained with the aid of Chalkley ratios. Nucleolar and cytoplasmic RNA concentrations were calculated from cytophotometric extinction ($E_{540\text{ m}\mu}$) measurements. Data were expressed as grains/unit area, grains/unit area/concentration (or specific activity) and grains/total structure. In the liver parenchymal cells of thioacetamide-treated rats, the nucleolus shows vast increases in volume, RNA content, and grain count/total structure, 14-fold, 25-fold, and over 30-fold, respectively. The nucleus increases 2-fold in volume and about 3-fold in total grain count. Cytoplasmic volume increases only 20 per cent and displays a total grain count about equal to that in the control. The time course of incorporation curves for nucleolus and non-nucleolar nucleus (NNN) contain 2 distinct turnover fractions, rapid and slow. Both fractions were increased after thioacetamide treatment but remained proportional to those of controls. The unique stimulated RNA turnover in the nucleus and nucleolus, coupled to a "normal" turnover in the cytoplasm, suggests that this nuclear-nucleolar loss of label does not represent an exclusive passage of formed nuclear RNA to the cytoplasm.

INTRODUCTION

The use of chemical compounds which experimentally alter or exaggerate some aspect of nucleoprotein metabolism has led to new insights into nuclear-cytoplasmic interactions (16, 27, 30, 40). It was hoped that such an approach might help to clarify some functional roles of nucleolar RNA in relation to other cellular RNAs. Thioacetamide

(CH_3CSNH_2)(TA), the compound used in this study, induces a rapid and specific effect on the nucleoprotein metabolism of liver parenchymal cells. Within a few days of treatment, the nucleolar volume and RNA content increased to enormous proportions, the nuclear volume and RNA concentration doubled, while the cytoplasmic RNA

concentration decreased (17, 18, 22). Biochemical studies on TA-treated liver have indicated that the amounts of nuclear RNA and protein and the levels of RNA and protein of the cytoplasmic "sap" were increased while microsomal and mitochondrial RNA and protein were depressed (2, 22). A pattern of enlarged nucleoli, with a concomitant increase in cytoplasmic basophilia, is typical of cells stimulated toward increased protein synthesis (3, for review). In contrast, thioacetamide-treated liver cells have enlarged, dense nucleoli, but contain diffuse and pale cytoplasmic basophilia (17, 18).

Although little is known about the mechanism of action of this compound, its effects on the RNA metabolism and chemomorphology of the liver cell have raised some interesting questions. Does thioacetamide, or a metabolite of this compound, stimulate nuclear synthesis and/or RNA breakdown? Does the drug block the transfer of nuclear RNA to the cytoplasm? How are the deranged RNA metabolism of the nucleolus and the NNN related to the changes observed in the cytoplasm? The results of this study, using H^3 -cytidine and high resolution radioautography, have revealed that both nucleolar and NNN RNA syntheses were increased (21), while isotope incorporation into cytoplasmic RNA was similar to that in control levels.

MATERIALS AND METHODS

Male rats of the Sherman strain, weighing 60 to 80 gm, were fed a diet of ground purina chow containing 0.04 per cent thioacetamide for 2 weeks. Each animal was injected intraperitoneally with a single dose of 200 μ c of tritiated cytidine (Schwarz Bioresearch Labs. Inc., Mount Vernon, New York) per 100 gm body weight. Animals were sacrificed in pairs, a thioacetamide-treated rat and a control rat, at intervals of $\frac{1}{2}$, 1, 2, 3, 5, 8, 12, 15, 18, and 24 hours after the injection of the tritiated cytidine. Thioacetamide is rapidly metabolized (17), and hence in order to maintain the maximum drug effect, an additional subcutaneous injection (5 mg/100 gm body weight) was administered 4 hours prior to isotope injection.

Excellent morphological preservation was obtained by rapidly freezing small pieces of liver in a liquid propane-isopentane mixture (1:1) cooled with liquid nitrogen to temperatures lower than -170° C, dehydrating in acetone at -50° C and postfixing at the same temperature in 2 per cent OsO_4 dissolved in anhydrous dimethylformamide for 1 to 2 hours, then washing in acetone at room tem-

perature and embedding in paraffin (25). Additional samples of liver were directly fixed in acid-alcohol mixture (1:3). Paraffin-embedded tissues were cut at appropriate thicknesses.

AR-10 stripping film, used in the conventional way, was placed over 2- and 4- μ sections and exposed for a length of time that resulted in comparable grain densities in both experimental and control tissues (36). These exposures were also within the range in which grain counts were proportional to exposure. Differences in density of intracellular organelles affect the range of β -particles (Maurer, W., and Primbsch, E., in press). Because of this, 2- μ sections were used almost exclusively for grain counts in both normal and thioacetamide-treated tissues, thereby insuring a high proportion of cut nucleoli in direct contact with the emulsion and eliminating self-absorption due to overlying chromatin. Moreover, even if the range of β -particles is restricted in the denser nucleoli of thioacetamide-treated tissues, correction for this would increase further the already vast differences between the experimental and control conditions. Comparison of these grain counts gave a general order of magnitude rather than an absolute measure of the number of molecules of precursor incorporated.

The following extractions were routinely performed: (1) 5 per cent trichloroacetic acid (TCA) at 2-4 $^\circ$ C was used for the removal of acid-soluble nucleotides. (2) 0.6 mg ribonuclease (Worthington Biochemical Co., Freehold, New Jersey)/ml distilled water adjusted to pH 6.5 at 37 $^\circ$ C for 2 hours, for RNA. (3) 0.6 mg deoxyribonuclease (Worthington)/ml of a 0.003 M $MgSO_4$ solution at 37 $^\circ$ C adjusted to pH 6.0 for 2 hours, for DNA. (4) 5 per cent TCA at 90 $^\circ$ C for 5 minutes, for the removal of all nucleic acids. The efficiency of these extractions was tested by grain counts and appropriate stains, azure B at pH 4.0 and the Feulgen reaction (41).

Cells containing a single nucleus and nucleolus were selected for the counts used in the figures. This population of cells was selected in order to increase the homogeneity of control and treated groups for statistical purposes. Grain counts were made over the nucleolus, NNN, and the cytoplasm. Grains falling on the edge of the nucleolus were included in nucleolar counts. Grains falling on the nuclear membrane were included in cytoplasmic counts.¹ A photograph was taken of each cell counted and enlarged in printing such that each cellular component could be readily outlined and then cut out. The relative areas of the components were

¹Morphological observations have shown that Palade granules are often associated with the outer surfaces of the nuclear membrane.

obtained by weighing the cutouts, and the data were expressed as grains/unit area. It was found that cuttings of equal unit volumes from successive sheets of Kodak enlarging paper was highly reproducible, yielding variations of less than 5 per cent.

Cytophotometric measurements of nucleolar and cytoplasmic RNA concentrations were made at 540 m μ on azure B-stained, DNase-treated preparations, whose thicknesses were determined by folds in the section (35). Single plugs were used to measure

formula

$$V = \frac{4}{3}\pi a^2 b,$$

where $a = \frac{1}{2}$ the smaller diameter and $b = \frac{1}{2}$ the larger diameter (17). Cytoplasmic volumes were determined by the Chalkley method (6).

Total number of grains for the whole nucleolus, non-nucleolar nucleus, and cytoplasm were calculated by multiplying the grains/unit area by the volume.

TABLE I
Nuclear, Nucleolar, and Cytoplasmic Volumes, Relative RNA Concentrations, Total RNA Amounts, and Total Grain Counts of Normal and Thioacetamide-Treated Liver Cells

Normal	Volume	Relative RNA conc. $E_{540m\mu}/\mu^3 \pm SE$	Relative total amt. RNA \ddagger in arbitrary units	Total incorporation of H $_3$ at the maximum \S
Nucleus	$328 \mu^3$	—	—	360.0 (2 hrs.)
Nucleolus	$5.5 \mu^3 \pm 0.85 SE$	0.150 ± 0.018	0.830	44.0 (2 hrs.)
Cytoplasm¶	$1600 \mu^3$	0.083 ± 0.015	133.0	1440.0 (15 hrs.)
<u>Thioacetamide</u>				
Nucleus	$711 \mu^3$	—	—	1420.0 (5 hrs.)
Nucleolus	$81.5 \mu^3 \pm 10.6 SE$	0.196 ± 0.02	16.0	1260.0 (5 hrs.)
Cytoplasm¶	$1990 \mu^3$	0.061 ± 0.009	121.4	1250.0 (15 hrs.)

* Each value in arbitrary units represents mean of 50 measurements obtained from animals at different times after isotope injection.

‡ Obtained by multiplying relative RNA conc. by volume.

§ Grain count/unit area \times volume. The sampling time in hours after isotope injection is given in parenthesis.

|| Average nuclear volume for the entire liver, obtained by multiplying class frequency by nuclear volume for each DNA class.

¶ Obtained with Chalkley ratios.

extinctions in nucleoli. Four plugs were used for the cytoplasm of each cell; the results were averaged and standard errors were calculated for the means. A comparison was made between the two wavelength method, which corrects for distributional error, and this plug method applied to the cytoplasm of normal liver cells. The results have shown that differences were of the order of only a few per cent (Swift, H., Rasch, E., and Kleinfeld, R., unpublished).

Specific activity (*i.e.* grains/unit area/RNA concentration) was calculated for nucleolar and cytoplasmic RNA.

Nucleolar and nuclear volumes were determined by direct measurement of whole uncut nuclei and nucleoli, and then calculations were made with the

Total amounts of nucleolar and cytoplasmic RNA were calculated by multiplying the mean $E_{540m\mu}$ by the volume (34).

RESULTS

Thioacetamide primarily affects cells of the central vein area (Fig. 5). These affected cells contain enlarged, dense nucleoli and a pale, diffuse cytoplasmic basophilia which contrast sharply with the relatively normal morphological appearance of cells in the portal area. Grain counts in the cytoplasm of cells in this latter area were markedly higher than in the former area (Figs. 10 and 11). After rats had been fed for 14 days a diet contain-

TABLE II
Nuclear Volumes and DNA Class Frequency in Normal and Thioacetamide-Treated Liver

	Normal liver		Thioacetamide-treated liver	
	Nuclear volume \pm SE	Frequency	Nuclear volume \pm SE	Frequency
	μ^3	%	μ^3	%
Diploid	170 $\mu^3 \pm$ 10.5	65.0	397 $\mu^3 \pm$ 18.7	58.0
Tetraploid	352 $\mu^3 \pm$ 19.1	34.0	859 $\mu^3 \pm$ 47.5	43.0
Octaploid	656 $\mu^3 \pm$ 43.5	1.0	1212 $\mu^3 \pm$ 61.0	0.4

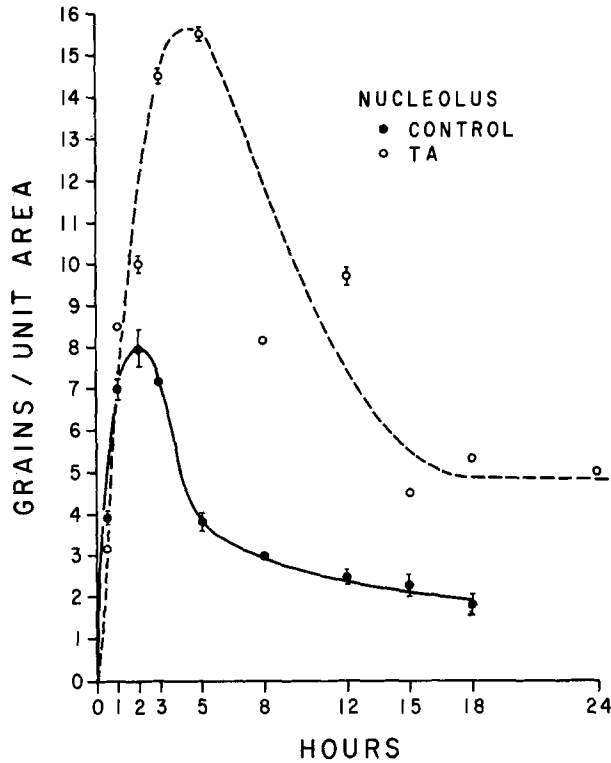


FIGURE 1 Changes in grain concentration with time, after a pulse labeling with tritiated cytidine, in the nucleolus of control and thioacetamide-treated parenchymal cells. Vertical lines represent standard errors; where they are lacking, SE falls within the symbol.

ing thioacetamide, 50 to 85 per cent of the liver cells in each animal revealed typical morphological changes induced by the compound. In the present study, the quantitative measurements used for the tables and curves were limited to cells of the central vein area. The data, therefore, do not represent the average cellular response to thioacetamide, but rather describe the maximally affected cell population. This becomes significant when comparisons are made with biochemical studies, reported in the literature, in which total liver homogenates were used.

Measurements of nucleolar, nuclear, and cyto-

plasmic volumes and of nucleolar and cytoplasmic RNA concentrations were determined from liver samples taken at different hours after isotope administration. The mean value for each parameter shows no significant variation with time, indicating that a relatively steady state is maintained at least over the 24-hour period during which liver samples were removed for study. The mean values obtained for control and treated animals are shown in Table I. After 2 weeks of thioacetamide administration, the nucleolar volume has increased 14-fold and RNA concentration has increased by $\frac{1}{8}$, resulting in a net 25-fold increase in

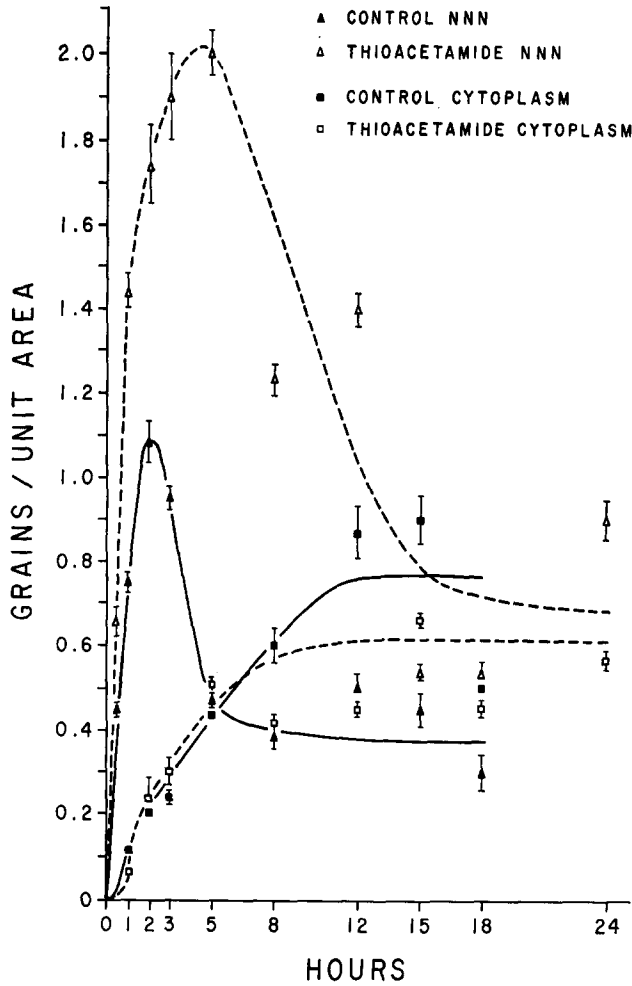


FIGURE 2 Changes in grain concentration with time in the NNN (non-nucleolar nucleus) and cytoplasm of control and thioacetamide-treated parenchyma after a pulse labeling with tritiated cytidine. Vertical lines represent standard errors.

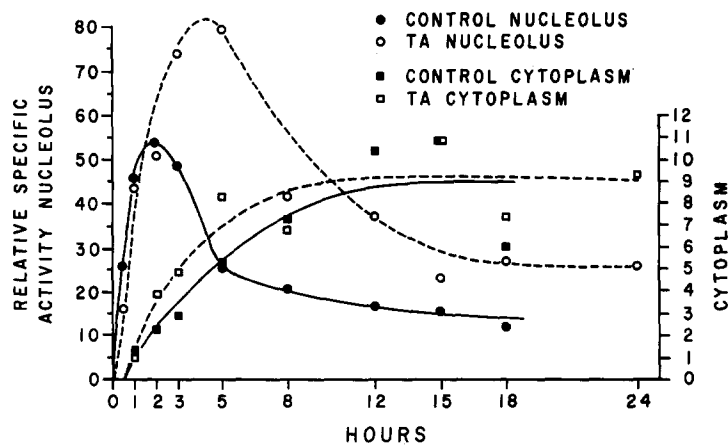


FIGURE 3 Changes in the relative specific activity (grains/unit area/ $E_{340 m\mu}$) with time, for the nucleolus (left) and cytoplasm (right) in normal and thioacetamide-treated parenchyma.

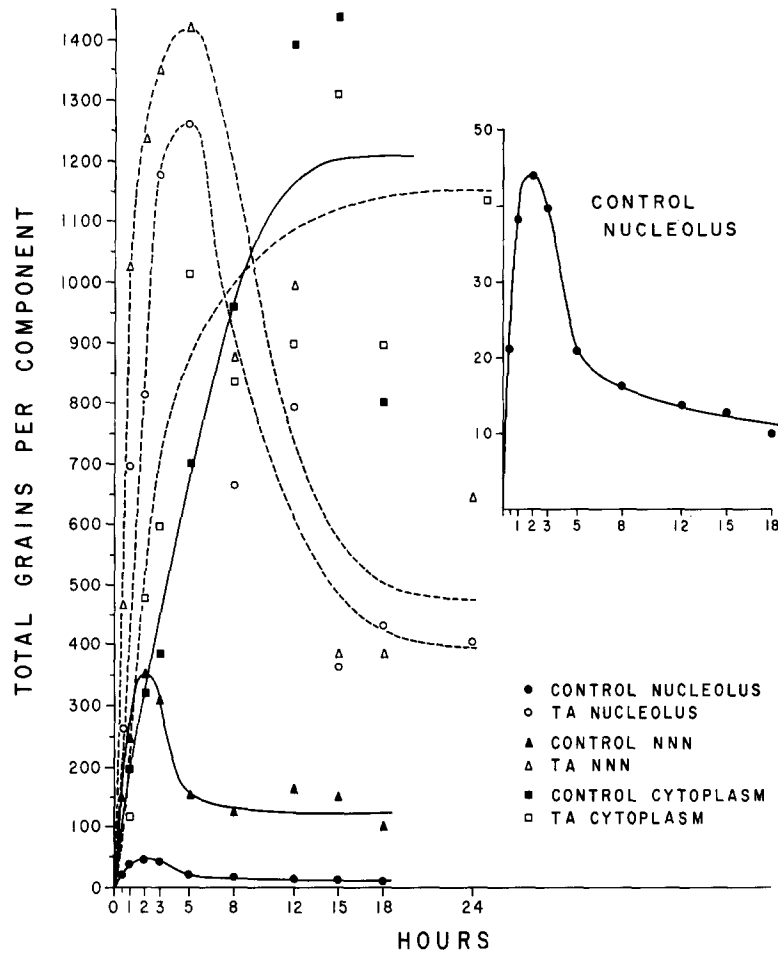


FIGURE 4 Changes in total grain count/component (grains/unit area \times volume of component) with time, in the nucleolus, NNN, and cytoplasm of control and thioacetamide-treated parenchyma. The insert depicts, in an expanded form, changes in the control nucleolus.

total amount of nucleolar RNA. Nuclear volume doubles for the three DNA classes (diploid, tetraploid, and octaploid) of nuclei, while the frequency distribution displays little or no change (Table II). The cytoplasmic volume of the affected cells increases approximately by $\frac{1}{4}$ so that the total amount of RNA is about equivalent to that in the normal cells.

Figs. 1 and 2 show the time course of incorporation of tritium into nucleolar, non-nucleolar nuclear, and cytoplasmic RNA per unit area, following a single injection of tritiated cytidine. Incorporation first appears in nucleoli and NNN (Figs. 6, 7), and after a lag of about 1 hour it then appears in the cytoplasm. The nucleoli at all times revealed both a greater grain density and a higher

rate of turnover than did the other structures. In nuclei containing more than one nucleolus, the several nucleoli are equally labeled. Maximum concentration of grains in nucleoli and NNN is reached at about 2 hours in the control group and at about 3 to 5 hours in the thioacetamide group (Fig. 8). Grain concentration at the maximum is 2-fold higher in the treated cells. Grain concentration in nucleoli is about the same in both normal and treated cells up to the 1st hour after H^3 -cytidine injection, after which it rapidly increases in the treated cells, in contrast to the controls. The time course of incorporation of H^3 cytidine and the maximum concentration of grains over cytoplasmic RNA were similar in both groups (Figs.

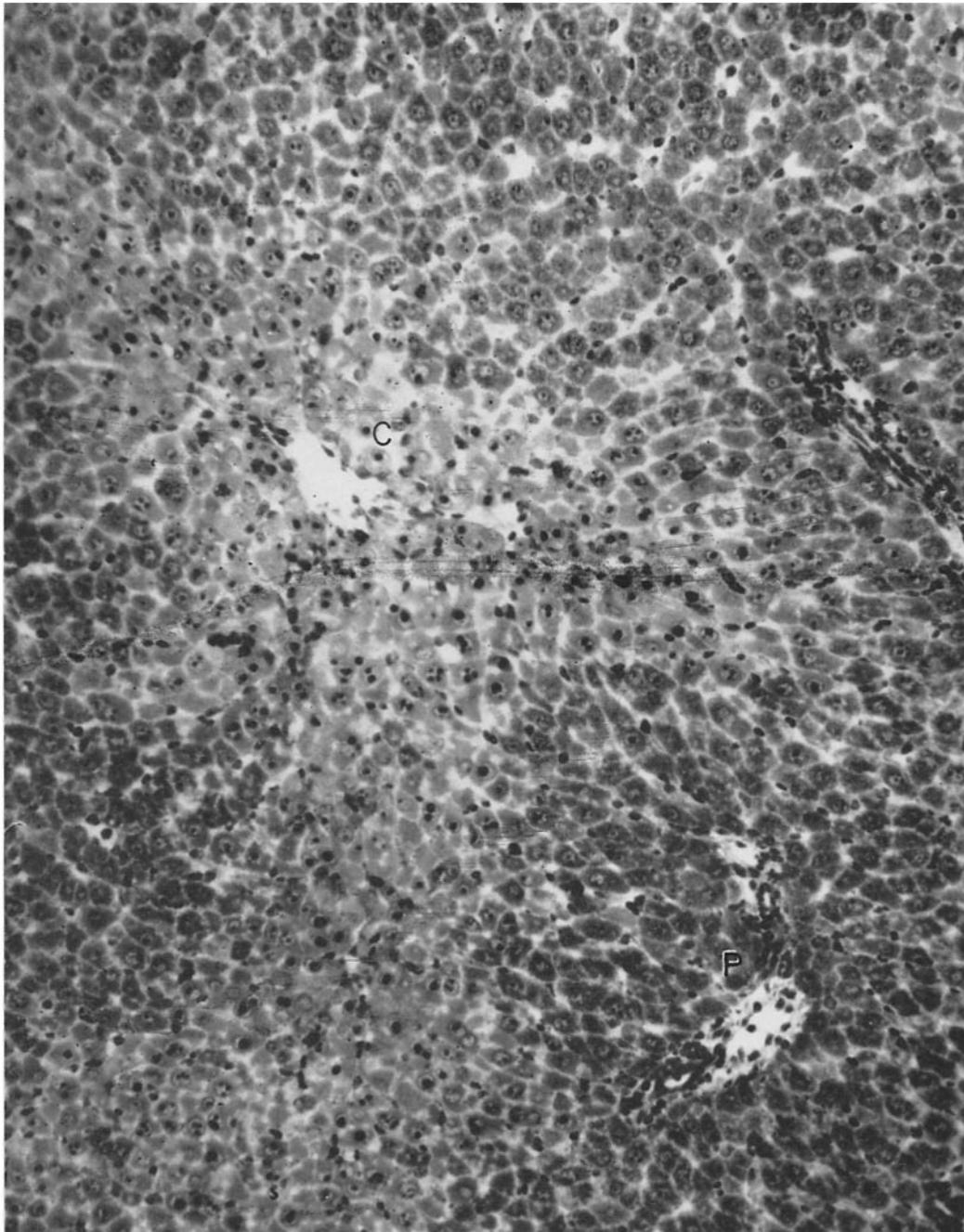


FIGURE 5 A low power view of thioacetamide-affected rat liver. Lightly stained areas around the central vein contain cells with greatly enlarged, dense nucleoli and decreased cytoplasmic basophilia. In the more deeply stained portal areas, cells contain smaller nucleoli and increased cytoplasmic basophilia. Azure-B stain. About $\times 200$. *C*, central vein area; *P*, portal vein area.

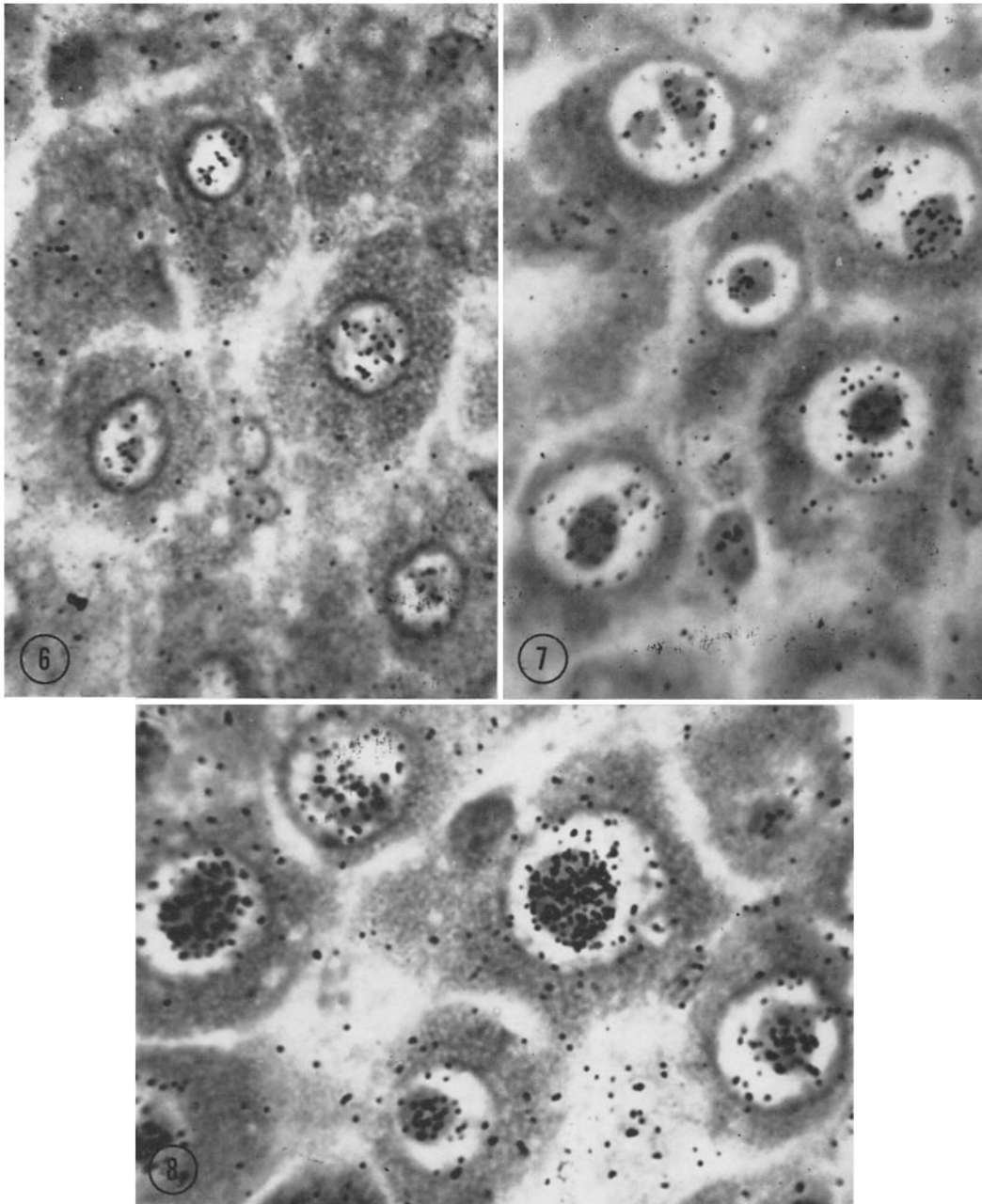


FIGURE 6 Control liver 1 hour after tritiated cytidine injection. Label almost exclusively localized in nucleoli and nuclei. Azure-B stain. About $\times 1800$.

FIGURE 7 Thioacetamide-affected liver 1 hour after tritiated cytidine injection. Label almost exclusively localized in nucleoli and nuclei. Azure-B stain. About $\times 1800$.

FIGURE 8 Thioacetamide-treated liver 5 hours after cytidine injection, showing maximum nucleolar labeling and some cytoplasmic labeling. Azure-B stain. About $\times 1800$.

9 and 10).² This occurs despite the fact that maximum labeling of NNN and nucleolus in treated cells is reached several hours later than in controls.

The *relative specific activities* of nucleolar and cytoplasmic RNA are plotted as a function of time in Fig. 3. The maximum specific activity of nucleolar RNA in treated cells is about 1.5 times that of the controls, while no difference is found in the specific activities of cytoplasmic RNA in the 2 groups.

The rapid loss of isotope from both nucleoli and NNN to approximately $\frac{1}{3}$ their peak values at 2 to 8 hours in the normal and 5 to 12 hours in treated material is followed by a much slower rate of loss during the next 12 hours. This may be interpreted as representing two metabolically distinct fractions, one with a rapid rate of turnover and one with a slow rate of turnover. After thioacetamide treatment, both "rapid" and "slow" fractions are increased proportionally in relation to those in the controls. An attempt to dilute the counts of the slow fraction by injecting carrier (non-radioactive cytidine in $10\times$ the amount injected as isotope, 41.4 mg/rat as opposed to 4.14 mg/rat) 12 hours after isotope injection was not effective. The nucleolar radioactivity in samples taken 12 hours after carrier injection was not significantly lower than control samples taken 12 and 24 hours after isotope injection without the carrier.

The *total grain counts* of each cellular component as a function of time are plotted in Fig. 4 (grain counts per unit volume multiplied by total volume). This expresses the relative rate of isotope incorporation per component and is particularly informative when comparing normal and experimental systems in which volumes and total RNA amounts are altered.

Although the specific activity of nucleolar RNA at the maximum in the treated group is only 1.5 times that of the controls, total nucleolar labeling is 30 to 40 times higher than the controls. The relative rate of isotope incorporation *per nucleolus* for the 1st hour is 30 grains/hour in controls and 693 grains/hour in the thioacetamide-treated cells; more than a 20-fold increase in the rate of incorporation occurs.

A rough estimate of the rate of loss of labeled nuclear RNA for the whole organelle may be made by calculating the ratios of the number of grains

² When correction was made for differences in exposure time, grain concentration in cytoplasm was about the same in control and treated cells.

lost per hour, during the period of rapid decline, to the maximum value. The rate of loss of grains during the 2- to 5-hour period in the control group is 7.7 grains/hour, or a loss of 17.5 per cent of the maximum isotope incorporation. In the treated group, the rate of loss of grains during the 5- to 8-hour period is 200 grains/hour and represents 15.5 per cent of the maximum radioactivity. Although nucleolar RNA loss occurs at a greater rate in thioacetamide-treated material, it represents a similar per cent of the total radioactivity.

When the total numbers of grains per component are compared at their respective maxima (Fig. 4), it is noted that in the control cell the cytoplasm contains 30 times the radioactivity of the nucleolus and about 4 times the activity of the NNN. In thioacetamide-treated cells, the cytoplasm has an over-all isotope incorporation similar to that of control cytoplasm (*i.e.*, approximately 1,000 grains) and contains only 0.8 the radioactivity of the nucleolus and 0.7 the activity of the NNN. The ratio of total grains for nucleus:cytoplasm, at their respective maxima, is approximately 1:3 for controls and 3:1 for treated cells.

In the control cell, the decrease in grain count that occurs between 2 and 18 hours for the total nucleus (nucleolus and NNN) is approximately 300 grains, which represents only $\frac{1}{4}$ of the total grain count of the cytoplasm at its maximum. In the treated cell, the decrease in grain count that occurs between 5 and 18 hours for the total nucleus is approximately 1,800 grains, which is 1.8 times more than the total maximum grain count associated with the cytoplasmic component. These results clearly indicate that the loss of nucleolar and non-nucleolar nuclear RNA counts in liver cells of thioacetamide-treated rats does not represent solely a simple passage to the cytoplasm.

DISCUSSION

The general picture which has emerged from recent radioautographic studies of RNA metabolism in intact cells indicates that: (a) nucleolar RNA synthesis is at least partially independent of non-nucleolar nuclear RNA synthesis (23, 24, 32, 37); (b) the major portion of cytoplasmic RNA is derived from RNA which is synthesized in the nucleus (1, 9, 10, 26-29, 32, 37); (c) breakdown of intranuclear RNA occurs, and some portion of this material may be involved in cytoplasmic RNA synthesis (7, 12-14, 23, 39).

There are differences in the patterns of in-

corporation, following a pulse exposure to labeled precursors, among the several organisms studied. For example, in *Drosophila* salivary gland cells, the pattern of incorporation in the nucleolus, following a pulse exposure to labeled precursors is distinct from that of the NNN and cytoplasm (23). In mammalian cells, non-nucleolar nuclear incorporation and nucleolar incorporation seem to occur at the same time, and the shape of their curves are similar (1, 8, 20).

Two metabolically distinct RNA fractions have been found in both nucleoli and NNN of rat liver cells, a fraction with a rapid rate of turnover and one with a slow rate of turnover (20, 37). The present study shows that in normal rat liver the ratios of the rapid to the slow fractions are approximately 3:1 for both nucleoli and NNN. In the treated liver, the 3:1 ratios are maintained despite the increase in turnover. This means that not only are increased amounts of nuclear RNA being synthesized, but also increased amounts are being lost. In contrast to this increased total labeled activity of the nucleus, the cytoplasm maintains an apparently "normal" level of cytidine incorporation. This rapid turnover fraction in the treated tissue could not all be molecular RNA *en route* to the cytoplasm, but may, in part, represent intranuclear degradation. This may also occur in the normal tissue. Several authors have presented evidence for intranuclear degradation of RNA to acid-soluble compounds (7, 12, 13, 39). It has been suggested that the rapid turnover of nucleolar RNA in *Drosophila*, termed "replacement RNA," may have a unique function which is accomplished upon degradation of the molecule within the nucleus (23). There is evidence which suggests that a portion of the degraded products of nuclear RNA may move to the cytoplasm to serve as precursors for cytoplasmic RNAs (7, 11, 39). Recent experiments with ameba, however, again point to

macromolecular RNA as being the unit which is transferred from nucleus to cytoplasm (9).

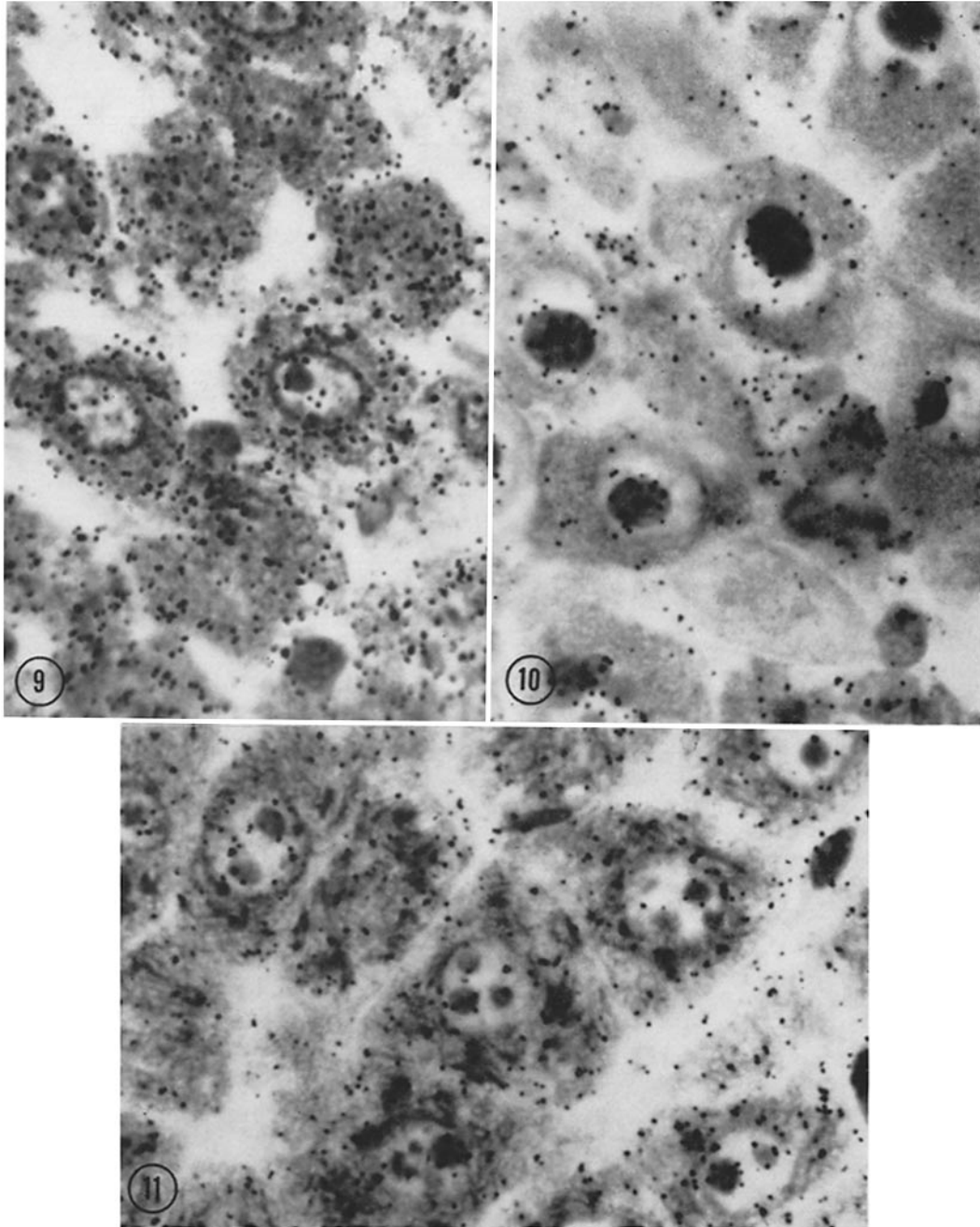
Four different nuclear RNAs have been characterized on the basis of their physical properties (33). A rapid turnover fraction suggestive of a "messenger" RNA has been described (15). Rapid and slow turnover fractions which have been reported in a few nuclear RNA studies (20, 37) and also described in this study indicate metabolic diversity. As discussed above, there is a rapid incorporation and loss of exogenous precursor in the rapid turnover fraction. In contrast, there is a relative metabolic stability in the slow turnover fraction. This was demonstrated, in the present study, by the fact that carrier did not dilute the label in this fraction. This may reflect a preferential utilization of intracellular intermediates which are not in competition with carrier nucleotides. That a preferential utilization of intracellular intermediates may occur has been described in HeLa cells (7). The heterogeneous nature of the nuclear RNAs and the diversity of functions attributed to the nucleus may make inadequate any simple hypothesis of nuclear RNA metabolism.

Changes in nucleolar RNA concentration have classically been associated with parallel changes in cytoplasmic RNA (3, for review). In regenerating rat liver, the increased rate of nucleolar RNA synthesis was accompanied by a parallel increased incorporation of isotope into cytoplasmic RNA (20). Suppression of nucleolar RNA synthesis by UV activation (26) or by actinomycin inhibition (27) is accompanied by a suppression of cytoplasmic RNA synthesis. However, in experiments with 8-azaguanine the labeling of the nucleolus was increased, while that in the cytoplasm had decreased. This result was interpreted as reflecting an interference with the transfer of RNA from the nucleolus to the cytoplasm, thereby causing an accumulation of RNA in the nucleolus (27). In-

FIGURE 9 Control liver 12 hours after tritiated cytidine injection. Label high in cytoplasm and low in nucleus. Azure-B stain. About $\times 1800$.

FIGURE 10 Thioacetamide-treated liver 12 hours after cytidine injection, *central vein area*. Cytoplasmic label only moderately high (when grain count is multiplied by 3, the correction for exposure time, the final value is equal to that in controls). Azure-B stain. About $\times 1800$.

FIGURE 11 Thioacetamide-treated liver 12 hours after tritiated cytidine injection, *portal area*. Cytoplasmic grain count higher than that in central vein area. Compare with Fig. 10. Azure-B stain. About $\times 1800$.



creased nucleolar masses accompanied by decreased cytoplasmic basophilia have been reported in protein deprivation studies on rat liver (33). In thioacetamide-treated rat liver, total incorporation of isotope into the nucleolus is found to be about 30-fold greater than the control value. Incorporation into cytoplasmic RNA remains comparable to control levels. In this system, large amounts of nucleolar RNA are being synthesized and lost while the cytoplasmic RNA metabolism is not drastically altered, as determined by labeled cytidine turnover. However, alterations in morphological aspects of the cytoplasm were apparent. Changes in azure-B staining of cytoplasmic RNA were observed, and electron microscopic examination of these liver cells revealed changes in endoplasmic reticulum and Palade granules (31).

It is clear from the time course of incorporation curves that the label does leave the nucleolus. The low, apparently "normal" level of label in the cytoplasm of treated cells may be due to improper utilization of nuclear products and acid-soluble precursors from cytoplasmic pools. It cannot yet be ruled out that a portion of the labeled RNA observed in the cytoplasm may have resulted from independent cytoplasmic RNA synthesis. Recent evidence suggests this as a possibility (14). Since only larger molecular weight RNA units were preserved by techniques used in this study, smaller molecular weight units may have accumulated in the cytoplasm, but would have been lost during the processing of the tissues. An increase in soluble cytoplasmic RNA fractions after thioacetamide treatment has been described (2, 22).

Some observations were made on cytidine label uptake in the lesser affected, portal areas. It is apparent that these cells which contain smaller nucleoli and a more intensely basophilic cytoplasm also contain greater concentration of labeled RNA in the cytoplasm than was present in the cytoplasm of cells in the central vein area. The increased cytoplasmic label in these "borderline recovery" cells may either passively reflect an increased receipt by the cytoplasm of nuclear products in a utilizable form or may mean that this cytoplasm may be better able to actively utilize the already available labeled nuclear products.

A biochemical study of the phenol-soluble (p-RNA) and insoluble (r-RNA) RNA fractions of "nucleolar," nuclear, and cytoplasmic components of rat liver has been reported by Adams and

Busch, (2). They found increases in the amount of nuclear RNAs and cytoplasmic "sap" p-RNA in TA-treated liver. Lowered uptake of orotic acid- 2-C^{14} , during the first 30 minutes after injection, led these authors to suggest that thioacetamide suppressed the synthesis of nuclear RNA. This contrasts with the findings reported here. Apart from the fact that the biochemical data represented the average cellular response to the drug, whereas the maximally affected cell was described here, certain other points should be clarified. With more than a twofold increase in the content of nuclear RNA noted in the treated livers, the specific activities during the very early sampling times were a poor measure of the comparative rates of nuclear synthesis in treated and control livers. The isotope incorporation curve that Adams and Busch presented indicated that the incorporation in the treated cells had not reached a peak at 30 minutes and, indeed, was still rising at 60 minutes. This comment also applies to their specific activity curves for the nucleolus in which 5- and 15-minute sampling times were used. Judging from the shift in the maximum incorporation to a later time in the RNA of the nucleolus and NNN of the treated cells presented here, a more meaningful comparison between the radioautographic and biochemical results would have been possible had Adams and Busch extended their sampling times. It is interesting to note that the time of maximum incorporation into RNA of the NNN and nucleolus is readily affected by experimental modification of rat liver. Partial hepatectomy shifts the non-nucleolar nuclear and nucleolar maxima to an earlier time (19), while thioacetamide treatment shifts these maxima to a later time. The magnitude of incorporation in both cases is greater than in the normal. The incorporation in thioacetamide-treated livers is even greater than that in regenerating liver. The time course of incorporation of tritium into nucleolar and non-nucleolar nuclear RNA involves many factors, including precursor pool size, rates of synthesis, molecular size, and size of turnover fractions. It is not realistic, therefore, on the basis of the data available (4), to undertake an analysis of the kinetics involved.

It is conceivable that a metabolite of the drug interferes with enzyme systems associated with RNA metabolism. Interaction of catabolic and anabolic pathways of pyrimidine metabolism are involved in altered rates of RNA synthesis (5). The fact that thioacetamide does not interfere

with restorative growth following partial hepatectomy (20) indicates that, despite the imbalance in nucleic acid metabolism, the affected cell can carry out syntheses required for cell duplication. This suggests that thioacetamide treatment does not impair the basic control mechanisms of the cell.

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