

LABELING OF NUCLEOLAR RNA WITH OROTIC ACID-2-C¹⁴ IN LIVERS OF
NORMAL AND THIOACETAMIDE-TREATED RATS*

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Thioacetamide produces an increase in the size of the nucleoli of liver cells (4,10), which is accompanied by an increase in nucleolar RNA (3,5) and protein (7,8). Studies on subfractions of RNA of the cell components revealed that thioacetamide suppressed incorporation of orotic acid into nuclear RNA as a whole, but had little effect on its incorporation into the nucleolar RNA. Fractionation of the nucleolar RNA revealed the presence of two components; thioacetamide did not affect the turnover of one of these.

MATERIALS AND METHODS: Albino male rats (Holtzman Co.) were injected with thioacetamide (I.P., 1% in saline solution) in a dose of 50 mg per kg body weight for nine days. On the tenth day, 3.3 μ curies of orotic acid-2-C¹⁴ were injected intravenously. The same amount of isotope was injected into control rats. The animals were killed and the livers were excised at 5 and 15 minutes after injection of the tracer. The livers were homogenized in 0.25M sucrose containing 0.005M calcium (9:1, v:w). The subcellular components were prepared as described previously (1,2). A re-

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cently improved method was used for the isolation of nucleoli after disruption of the nuclei by sonication (9).

The RNA was extracted by the method of Kirby (6) as modified by Sibatani *et al* (12). From each cellular fraction, two RNA fractions were obtained: 1) RNA in the aqueous phase (p-RNA), and 2) RNA in the phenol-aqueous interphase (r-RNA). This last fraction has been postulated by Sibatani *et al* (11) to contain "messenger" RNA. The quantity of RNA was determined by the orcinol method. The samples were plated on stainless steel planchets at infinite thinness and counted in a thin window gas-flow counter (Nuclear Chicago).

RESULTS: The amount of RNA was approximately doubled in the nuclei, nucleoli, and cytoplasmic sap of livers of thioacetamide-treated animals. The amount of RNA in the mitochondria and microsomes was approximately half the value for normal liver. The greatest change was in the r-RNA of the nucleolus which increased 3.5 fold over the normal values.

The specific activities of the RNA fractions from normal livers are presented in Figs. 1 and 2. The highest specific activities were found in the nuclear and nucleolar RNAs both at 5 minutes (Fig. 1) and 15 minutes (Fig. 2) after injection of the labeled orotic acid. At 5 minutes (Fig. 1), the specific activity of nuclear r-RNA was the highest. At 15 minutes both the nucleolar r-RNA and the nucleolar p-RNA had approximately the same specific activities as the nuclear r-RNA.

The labeling of much of the RNA was markedly suppressed by thioacetamide (Fig. 3, Fig. 4). However, the specific activity of the p-RNA of the nucleoli was not different from normal values at 5 minutes (Fig. 3) and 15 minutes (Fig. 4). On the other hand, the labeling of the r-RNA of the nucleoli was suppressed by 20%

at 15 minutes (Fig. 4). By comparison, the labeling of the r-RNA of the nuclei was suppressed by 60%.

DISCUSSION: The data obtained in this study suggests the presence in the nucleus of four metabolically distinct RNA fractions. The p-RNA of the nuclei was very low in specific activity in nu-

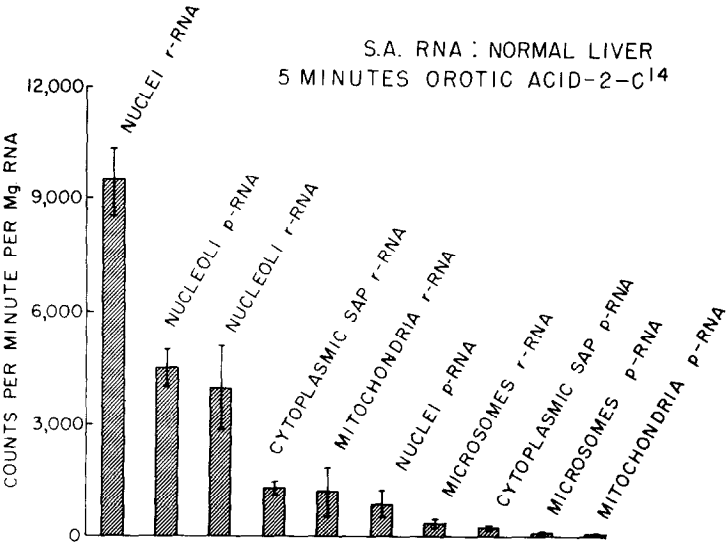


Fig. 1

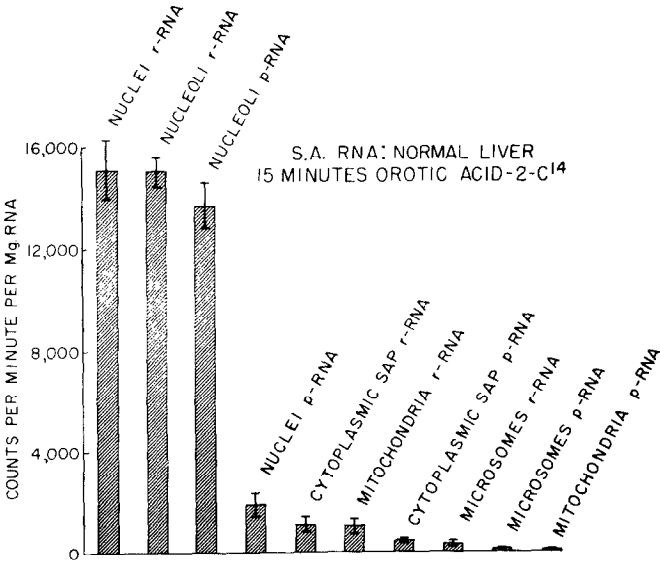


Fig. 2

clei of livers of both normal and thioacetamide-treated animals. The synthesis of the r-RNA of the chromatin, and that of the two RNAs of the nucleoli are apparently independent of one another although some decrease was noted in the specific activity of the r-RNA of the nucleolus in the livers of thioacetamide-treated rats.

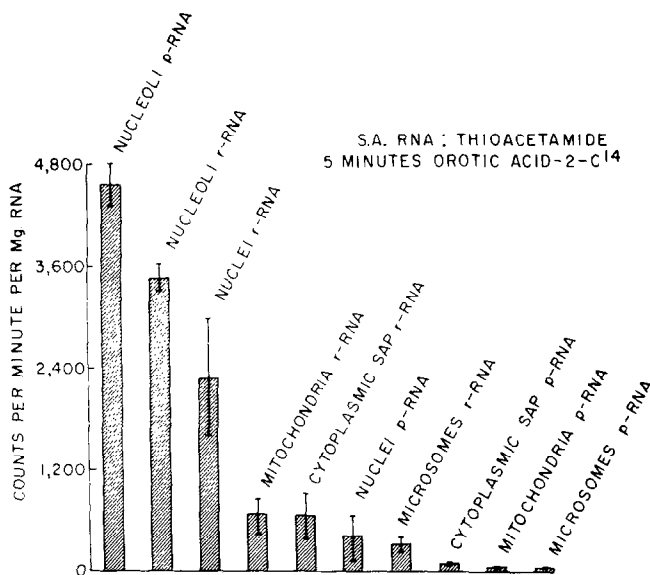


Fig. 3

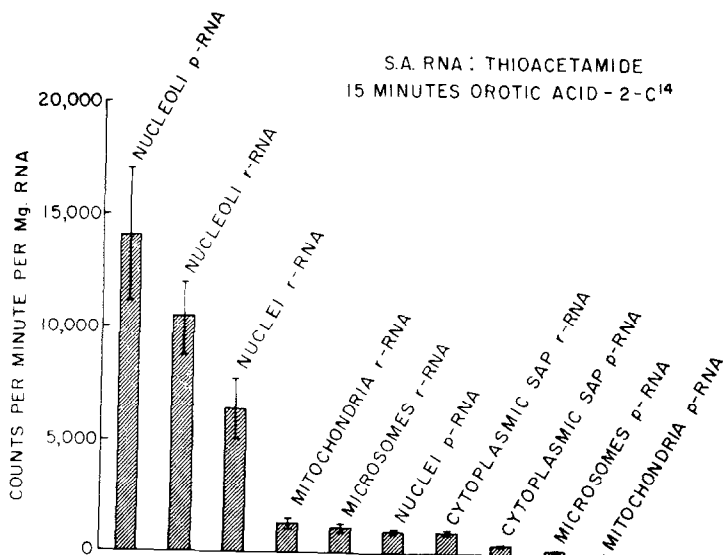


Fig. 4

Other evidence for the apparent independent synthesis of RNA of the nucleolus has arisen from the studies of Sirlin et al (13), who found that nucleoli of cells of salivary glands of *Smithia* larvae, treated with 4,5,6-trichloro-1- β -D-ribofuranosylbenzimidazole continued to label RNA in the absence of synthesis of chromosomal RNA; the suggestion has been made that the RNA synthesized is transfer RNA (13,14). The low rate of labeling of nuclear p-RNA is of interest in view of the suggestions of Sibatani et al (12) and Zubay (15) that the nucleolus may contain a primordial informational RNA.

SUMMARY: Evidence obtained by short time studies with orotic acid- -2-C^{14} as a tracer has shown the existence in liver cells of two metabolically distinct nucleolar RNAs which are differentiated from two extranucleolar RNAs (12). In normal livers, the RNA with the highest initial specific activity was the nuclear r-RNA. In livers of thioacetamide-treated animals, the RNA with the highest initial specific activity was nucleolar p-RNA. The nuclear p-RNA had the lowest specific activity in these studies. The data obtained suggest the presence of three RNA fractions in nuclei with high rates of turnover.

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