INHIBITION BY α-AMANITIN OF CHROMOSOMAL RIBONUCLEIC ACID SYNTHESIS IN RAT LIVER

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1. Introduction

Three RNA polymerases (EC 2.7.7.6) have been identified in mammalian nuclei: a nucleolar RNA polymerase I, and the extranucleolar polymerases II and III [1]. The product of RNA polymerase I is ribosomal (or ribosomal precursor) RNA [2] whereas the nature of the products of the two other polymerases is less defined. Widnell and Tata [3] observed that at high ionic strength (which stimulates the activity of RNA polymerase II) rat liver nuclei synthesize a 'DNA-like' RNA. According to Zylber and Penman [4] RNA polymerases II and III synthesize heterogeneous RNA. The latter results were obtained with the use of α-amanitin, a mushroom toxin which impairs the synthesis of RNA [5] by inhibiting specifically the activity of RNA polymerase II (for review see Fiume and Wieland, [6]).

A class of RNA molecules associated with chromatin and termed chromosomal RNA (cRNA) has been described [7–9], and Mayfield and Bonner [10] suggested that this cRNA originates from the transformation of heterogeneous RNA. On the other hand, the hypothesis has been formulated that this RNA may be a degradation derivative of tRNA (although Holmes et al. [11] reported that cRNA and tRNA have a different electrophoretic mobility), and the very existence of cRNA has been questioned [12–14]. Recently, a method has been described to purify cRNA [15] which according to the authors is the best suitable for animal tissues. The data reported above and the availability of this method prompted us to investigate the effect of α-amanitin poisoning on the synthesis of cRNA and of tRNA in rat liver. The results reported in this paper demonstrate that the synthesis of cRNA species is strongly and rapidly impaired in the liver of rats poisoned with α-amanitin.

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2. Materials and methods

Male Wistar rats weighing 120–130 g or 250–300 g were used. Partial hepatectomies were performed under ether anaesthesia by the method of Higgins and Anderson [16]. Animals were killed 24 hr after the operation. α-Amanitin (150 μg/100 g of body wt) was injected intraperitoneally 60 or 20 min and [6-^{14}C]orotic acid (60.8 mCi/mmole, 10 μCi/100 g of body wt) was injected intraperitoneally 20 min before killing the animals.

The livers were pooled until 10–20 g were obtained and used to extract chromatin according to the procedure of Marushige and Bonner [17] but omitting the pronase treatment. Chromosomal RNA was extracted from the chromatin and purified one time by chromatography on DEAE-Sephadex as described by Mayfield and Bonner [15]. The eluate from the column was collected in 1-ml fractions; the E_{260} was recorded and 0.4-ml samples of the fractions were mixed with 5 ml of ethylene glycol monomethyl ether and with 10 ml of scintillation fluid (0.01% 1,4-bis-(5-phenyloxazol-2-yl) benzene and 0.4% 2,5-diphenyloxazole in toluene) and the radioactivity was determined in a Nuclear-Chicago Mark II scintillation counter. The remainings of the fractions containing radioactivity were pooled, the radioactivity of the pool was measured again, and the RNA content

was determined as described by Munro and Fleck [18], and the specific activity of chromosomal RNA was calculated from these data. RNA was extracted also from samples of the whole liver homogenate, was determined according to Munro and Fleck [18], and its radioactivity was measured as described above. tRNA was extracted from rat liver 105 000 *g* supernatant by the method of Kirby [19] and appeared as a single band on polyacrylamide gel electrophoresis [11].

3. Results and discussion

The incorporation of [^{14}C]orotic acid into RNA extracted from whole rat liver homogenate was depressed after the administration of α-amanitin (table 1). The extent of the inhibition was consistent with the results obtained by Stirpe and Fiume [5] with mouse liver, and was approximately the same in the liver of growing and adult rats, but was somewhat less in regenerating liver.

The incorporation of [^{14}C]orotic acid into chromosomal RNA was also inhibited after administration of α-amanitin, and in all experiments to a greater extent than the synthesis of whole RNA. It is noteworthy that in adult rats the synthesis of chromosomal RNA was inhibited by approx. 70%, whereas the inhibition was less severe in the liver of younger rats and in regenerating liver. In the latter there was a higher labelling of chromosomal RNA, which is consistent with the results of Mayfield and Bonner [10]. Representative elution profiles of chromosomal RNA are shown in fig. 1, together with the radioactivity of the effluent.

The hypothesis that cRNA may derive from tRNA

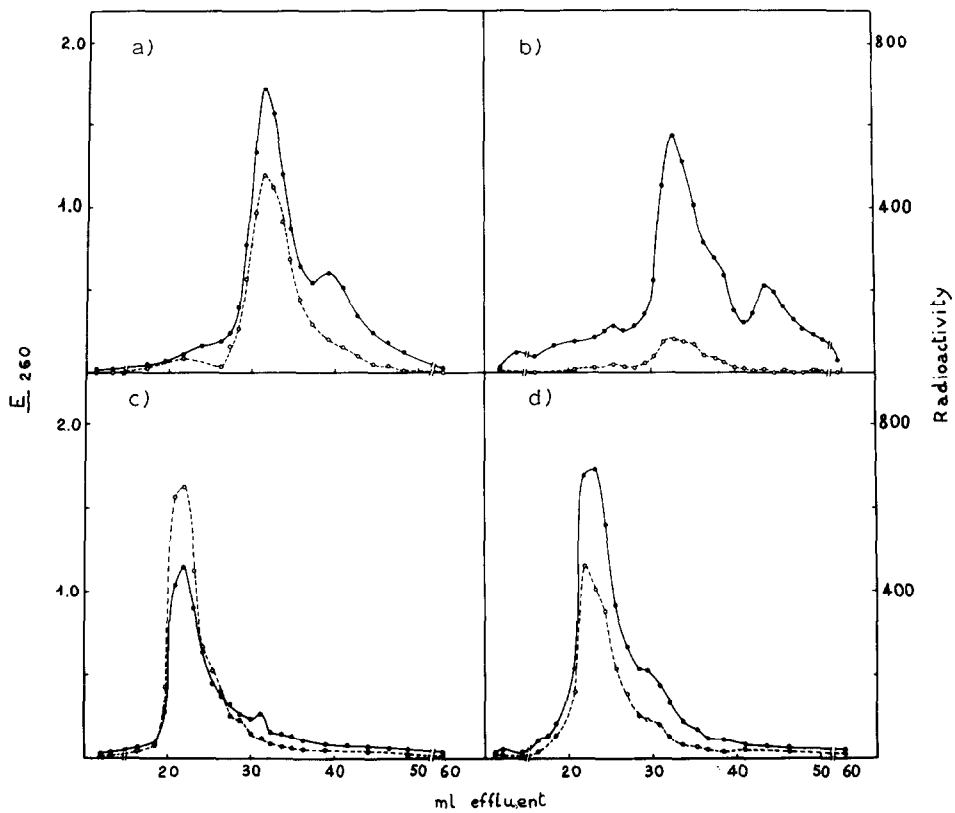

Fig. 1. Elution profiles of chromosomal RNA from DEAE-Sephadex. (*a*) Normal and (*b*) α-amanitin poisoned liver from 300 g rats (exp. 3 of table 1); (*c*) 24-hr regenerating and (*d*) 24-hr regenerating poisoned liver from 120 g rats (exp. 2 of table 1). (o—o—o) E_{260}; (o- - o- - o) radioactivity (cpm/0.4 ml).

Table 1
Effect of α-amanitin poisoning on the incorporation of [6-^{14}C]orotic acid into homogenate and chromosomal RNA.

Exp. no.	Animals	Specific activity of RNA			
		Homogenate RNA	Inhibition (%)	Chromosomal RNA	Inhibition (%)
1	Normal, 300 g	15.24		4.77	
	Poisoned, 300 g	8.72	43	1.78	63
2	Normal, 120 g	17.02		15.59	
	Poisoned, 120 g	11.73	31	7.80	50
	Partially hepatectomized, 12 g	14.78		18.03	
	Partially hepatectomized and poisoned, 120 g	8.71	41	7.34	59
3	Normal, 300 g	15.58		15.25	
	Poisoned, 300 g	6.98	55	2.88	81
4	Normal, 250 g	11.33		4.13	
	Poisoned, 250 g	6.59	42	1.73	58

Experimental conditions were as described in the text. The specific activity of RNA is expressed as cpm/mg of RNA divided by the acid-soluble radioactivity of the homogenate corresponding to 1 mg of wet liver.

Table 2
Effect of α-amanitin poisoning on the incorporation of [6-^{14}C]orotic acid into homogenate and transfer RNA.

Exp. no.	Animals	Specific activity of RNA			
		Homogenate RNA	Inhibition (%)	Transfer RNA	Inhibition (%)
1	Normal, 20 min saline	13.03		2.29	
	Poisoned, 20 min	6.49	50	1.55	32
	Poisoned, 1 hr	2.07	84	0.65	72
2	Normal, 20 min saline	14.45		5.19	
	Poisoned, 20 min	8.33	42	3.22	38
	Normal, 1 hr saline	10.30		5.28	
	Poisoned 1 hr	2.00	81	1.56	70

Experimental conditions were as described in the text. Rats, weighing about 300 g, were poisoned 20 min or 1 hr before killing. The specific activity of RNA is expressed as cpm/mg of RNA divided by the acid-soluble radioactivity of the homogenate corresponding to 1 mg of wet liver.

[12] and the doubts cast on the existence of cRNA as a separate class of RNA prompted us to study the effect of α-amanitin poisoning on the synthesis of tRNA. The results reported in table 2 demonstrate that the synthesis of tRNA was also inhibited, but to a lesser extent than the synthesis of total RNA.

Our results indicate that the synthesis of chromosomal RNA depends upon the activity of RNA polymerase II, assuming that the inhibition of this enzyme is the only direct effect of α-amanitin. The greater severity of the inhibition of chromosomal RNA synthesis, as compared with the synthesis of total RNA, suggests that this RNA may be or may derive from the product of RNA polymerase II. If one

considers that Zylber and Penman [4] reported that polymerase II and III produce high-molecular weight heterogeneous RNA, our results give some support to the hypothesis of a precursor–product relationship between heterogeneous and chromosomal RNA [14]. The fact that the synthesis of tRNA was less inhibited by α-amanitin suggests that cRNA is not a derivative of tRNA. A great biological importance has been attributed to chromosomal RNA [10–15, 20, 21]. Experiments are in progress to ascertain, with the use of α-amanitin, the possible correlations between the synthesis of chromosomal cRNA and the synthesis of other RNA species, of protein and of DNA.

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