ON THE INHIBITION OF RNA SYNTHESIS BY ACTINOMYCIN

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Received January 22, 1962

In Neurospora crassa the growth-inhibitory effect of actinomycin C can be counteracted by adding DNA to the medium. RNA was found to be far less effective than DNA (Kersten et al., 1960). The reversal of inhibition is due to the formation of complexes between actinomycin and the nucleic acids in the medium (Rauen et al., 1960). Wheeler and Bennett (1960) observed an inhibition of nucleotide and nucleic acid metabolism by actinomycin.

In the following experiments Ehrlich ascites tumor cells (hypertetraploid cell line) were incubated for 1 hour under conditions as described previously (Harbers and Heidelberger, 1959 a). In this system, actinomycin-C_1 had a considerable inhibitory effect on the incorporation of 8-C^{14}-guanine into RNA purines. At higher concentrations the synthesis of RNA was completely blocked (Fig. 1). From the total activities in the acid-soluble fraction it can be seen that actinomycin did not prevent the formation of labeled nucleotides. On the contrary, actinomycin stimulated the incorporation of labeled guanine by the cells. Also, the total activity of the nucleoside di- and triphosphates increased as compared to the controls (Table 1). Therefore, the inhibition of RNA synthesis by actinomycin cannot be due to effects at the nucleotide level, but must be at the site of the polymerization process. Similar results were obtained using as a labeled precursor 2-C^{14}-uracil (Fig. 2). In both types of experiments some inhibition of the incorporation into DNA could also be observed (Fig. 1 and 2).

So far, the binding of actinomycin to DNA has been demonstrated in model experiments only. To investigate whether this phenomenon also occurs in the intact cell, Ehrlich ascites tumor cells were incubated with C^{14}-labeled actinomycin. From the results, it can be seen that actinomycin is in-

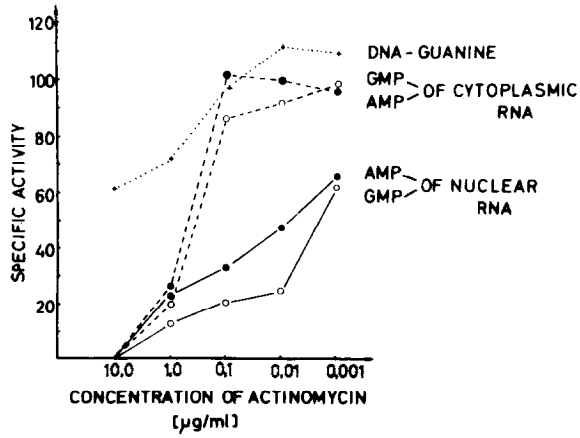

Fig. 1. The effect of actinomycin on the incorporation of 8-C^{14}-guanine into the purine nucleotides of RNA and into the guanine moiety of DNA. Per cent of control specific activities against concentration of actinomycin in µg/ml.

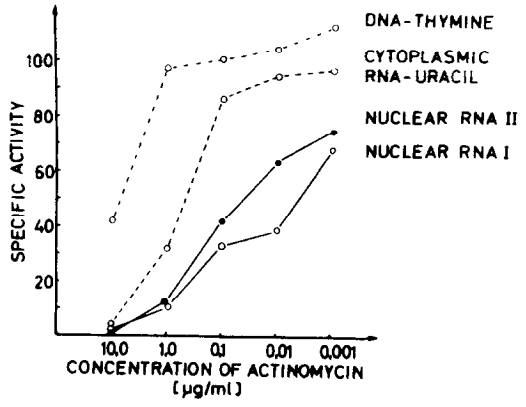

Fig. 2. The effect of actinomycin on the incorporation of 2-C^{14}-uracil into DNA-thymine and RNA-uracil. Per cent of control specific activities against concentration of actinomycin in µg/ml. The cell nuclei were fractionated as described by Allfrey and Mirsky (1957). RNA I = pH 7.1-soluble RNA; RNA II = pH 7.1-insoluble RNA.

corporated by the cells very rapidly. Within the cells more than 80 per cent of the C^{14}-actinomycin was found in the nuclei. After fractionating the nuclei, about 95 per cent of the labeled material was localized in the DNA containing fraction. No intensive metabolic degradation of actinomycin could be observed.

Table 1

Total C^{14}-content in the acid-soluble fraction and two of its sub-fractions of Ehrlich ascites tumor cells which had been incubated for 1 hour with 8-C^{14}-guanine. The 5'-guanosine-monophosphate (GMP) and the polyphosphates (containing C^{14}-labeled ADP, GDP, ATP, and GTP) were isolated by exchange chromatography. Activity in counts/min, measured in a windowless flow-counter.

Concentration of Actinomycin	Total Activity	GMP	Polyphosphates
Controls	354 000	31 000	112 200
0.001 µg/ml	426 000	39 000	151 000
0.01 µg/ml	443 000	54 000	188 000
0.1 µg/ml	467 000	57 000	193 000
1.0 µg/ml	523 000	59 000	231 000
10.0 µg/ml	568 000	74 000	262 000

Since the formation of complexes between DNA and actinomycin or its derivatives occurs only with compounds of high biological activity (Müller, 1962), and the complex formation could be demonstrated also in the living cell, it is concluded that RNA synthesis is prevented by actinomycin due to a partial occupation of the DNA template. Thus, only the DNA-dependent type of RNA synthesis should be sensitive to actinomycin. This hypothesis is supported by the observation that after short incubation (15 minutes), actinomycin inhibited only the incorporation of 2-C^{14}-uracil into nuclear RNA, but not into cytoplasmic RNA. Furthermore, actinomycin had no, or at high concentration, only little inhibitory effect on the incorporation of labeled orotic acid into cytoplasmic RNA in a rat liver homogenate system which does not contain DNA (Table 2).

Table 2

Conversion of 2-C^{14}-orotic acid into RNA-uracil after incubation of a fortified cytoplasmic fraction of rat liver for 1 hour. Conditions similar to those described by Schneider and Potter (1958). In this system nucleotides are attached to the end of RNA molecules which are already present (Harbers and Heidelberger, 1959 b), but the formation of some new RNA probably also takes place. Specific activity (counts/min per mg RNA phosphorus) in per cent of the controls.

Concentration of Actinomycin	Specific Activity
Controls	100
5.0 µg/ml	73
0.05 µg/ml	99

According to Reich et al. (1961) actinomycin D (= actinomycin-C_1) inhibits the formation of a DNA virus, while it does not influence the multiplication of the RNA-containing Mengo virus. Later these authors also came to the conclusion that actinomycin inhibits, in a specific way, the RNA synthesis which is DNA dependent (Reich, personal communication).

This work was supported by a grant from the Deutsche Forschungsgemeinschaft, Bad Godesberg, Germany. The hypertetraploid line of the Ehrlich ascites tumor was kindly supplied by Dr. Charles Heidelberger, Mc Ardle Memorial Laboratory, University of Wisconsin, Madison, Wisc., U.S.A.

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