EFFECTS OF ACROLEIN ON TRANSCRIPTION *IN VITRO*

Y. MOULÉ and C. FRAYSSINET
with the technical assistance of N. ROUSSEAU
Institut de Recherches Scientifiques sur le Cancer, 94-Villejuif, France

Received 30 March 1971
Revised version received 18 June 1971

1. Introduction

It is well known that unfiltered cigarette smoke has induced cytological and cytochemical lesions on mouse kidney cells in culture [1]. Inhibition of RNA synthesis occurs as early as 1 to 2 hr after exposure to the gas phase of unfiltered smoke. Furthermore, it has been suggested that acrolein, a main constituent of this phase, might be responsible for the observed cell damages [2]. This view is supported by recent results which show that a dose of 320 μg of acrolein per 100 g of body weight (that corresponds to the burning of four cigarettes) strongly inhibits RNA and DNA synthesis in regenerating rat liver [3].

Several possibilities might be considered to explain the inhibitory effect of the drug: impairment of the nucleotide pool, binding to the DNA, inactivation of specific enzymes, etc. As far as RNA synthesis is concerned the present results report the inhibition produced by acrolein on RNA polymerase activity in mammalian systems, as well as on bacterial enzyme; moreover, it is suggested that acrolein inhibits transcription by acting on the enzyme itself rather than on the DNA template.

2. Methods

Male Wistar rats (Commentry strain) were fasted 15 hr before killing by decapitation. The livers were quickly removed, chilled at 0° and homogenized in 9 volumes of 2.2 M sucrose (d = 1.28) containing 1 mM $MgCl_2$ or 1 mM $MnCl_2$. The nuclei were isolated according to Chauveau, Moulé and Rouiller [4]. Nuclei were usually kept at −50° without enzyme inactivation before being tested.

Various dilutions of freshly distilled acrolein were made in water and immediately used. The estimation of RNA polymerase activity was carried out as previously described [5] and the composition of the standard assays was indicated in the figures. When incubations were performed at high ionic strength, 0.02 ml of saturated ammonium sulphate solution adjusted to pH 8 with NH_4OH were added to the medium.

Purified RNA polymerase from *E. coli* was purchased from Miles Laboratories (Elkhart, Indiana, USA).

3. Results and discussion

Addition of acrolein to isolated nuclei synthesizing RNA-like products leads to an inhibition of their transcriptional ability. The inhibition appears directly related to the amount of acrolein added between a range of 40 to 400 μg (fig. 1). The decrease in polymerase activity is unchanged in regard to the divalent cation (whether Mg^{2+} or Mn^{2+}) used for isolating and incubating nuclei [5]. Moreover, inhibition of *in vitro* transcription may also be observed, although to a lesser extent, when incubations are performed at high ionic strength. Such results suggest that the two distinct RNA polymerase enzymes present in liver nuclei (which differ in requirement for optimal activity [5–8] and intranuclear localization [9, 10]) both appear to be impaired by acrolein; *in vitro* systems working with the purified mammalian enzymes

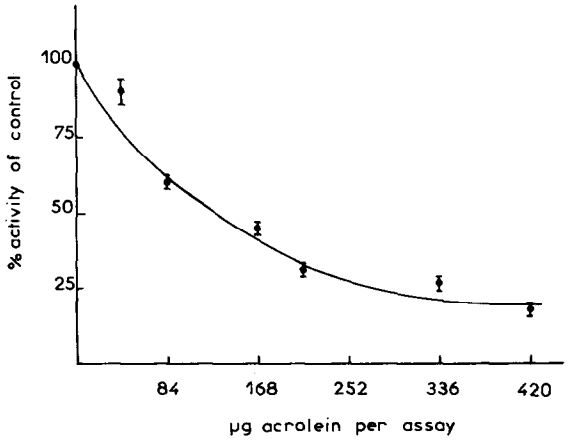

Fig. 1. *In vitro* effect of acrolein on transcription by isolated rat liver nuclei. The complete system contained in 0.30 ml: 40 μmole Tris-HCl buffer pH 9, 2 μmole $MgCl_2$, 0.25 μmole each of three unlabeled nucleoside-5'-triphosphates (ATP, GTP, UTP), 0.25 μmole of (8-^{14}C) CTP corresponding to 0.1 μCi, 0.05 ml of distilled water or acrolein solution, 0.2 ml of suspension of nuclei. Nuclei isolated from 10 g of liver were resuspended in 4.5–5 ml of 0.05 M tris-phosphate buffer pH 7.4 containing 0.01 M β-mercaptoethanol. Suspension of nuclei and acrolein solution were carefully mixed and kept for 10 min at 0°. The reaction was initiated by addition of nucleoside triphosphates and run in duplicate for 10 min at 37°. Determination of polymerase activity was carried out as previously described [5]. Values are mean results of 5 experiments.

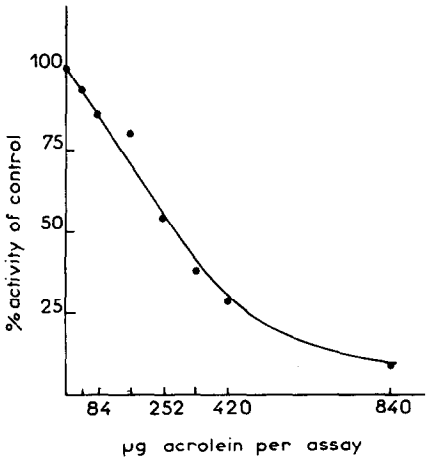

Fig. 2. *In vitro* effect of acrolein on transcription by RNA polymerase of *E. coli*. The complete system contained in 0.25 ml: 10 μmole Tris buffer pH 9, 0.25 μmole $MnCl_2$, 1.25 μmole $MgCl_2$, 3 μmole β-mercaptoethanol, 80 μg calf thymus DNA, 0.025 μmole each of three unlabeled nucleoside-5'-triphosphates (ATP, GTP, CTP), 0.025 μmole of (2-^{14}C) UTP corresponding to 0.09 μCi, 0.05 of distilled water or acrolein solution, 5 units of purified RNA polymerase of *E. coli*. The reaction was initiated by addition of enzyme preparation and run for 10 min at 37°. The control assay corresponds to the incorporation of 1816 ± 50 pmole (2-^{14}C) UTP.

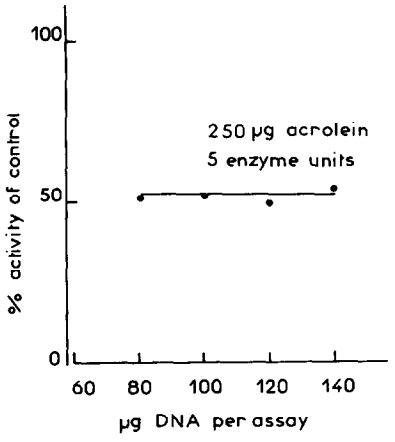

Fig. 3. Effect of progressively higher amounts of DNA on the extent of inhibition of transcription by acrolein (RNA polymerase of *E. coli*). Incubations were performed under the conditions described in the legend of fig. 2 in presence of DNA amounts as indicated on the graph.

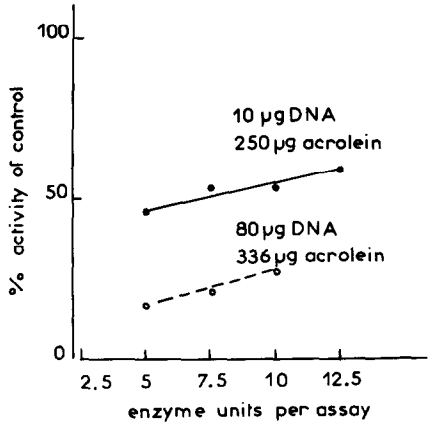

Fig. 4. Effect of progressively higher amounts of RNA polymerase of *E. coli* on the extent of inhibition of transcription by acrolein. Incubations were performed under the conditions described in the legend of fig. 2 in presence of amounts of enzyme as indicated on the graph. •——• addition of 10 μg of DNA that corresponds to a DNA concentration which is a limiting factor for the reaction. •- - - • addition of 80 μg of DNA that corresponds to a DNA concentration which is not a limiting factor for the reaction.

might confirm this view. However, the results agree with the fact that *in vivo* inhibition of transcription in liver occurs approximately to the same extent for nuclear and nucleolar RNA [3].

It must be pointed out that *in vitro* inhibition of transcription by acrolein is not limited to the eukaryotic system: bacterial RNA polymerase is also strongly inhibited by the aldehyde (fig. 2). This agrees with recent results which report that RNA synthesis was decreased in *E. coli* after addition of acrolein to the medium [11]. Further experiments performed in order to provide information regarding the site of action of acrolein suggest that it acts on the enzyme itself. The extent of inhibition is unaffected by the amount of DNA added in the assay, whereas addition of progressively higher levels of enzyme leads to a partial recovery of the transcriptional activity (figs. 3 and 4). Similar results have been published concerning the mechanism whereby α-amanitin inhibits *in vitro* transcription [8, 12].

Inhibition of RNA synthesis can be produced by various compounds, in particular, by many carcinogenic molecules. On account of its effects on *in vivo* and *in vitro* transcription in mammalian cells, it seems to us that the actual role of acrolein in pathological activity of cigarette smoke should be reconsidered.

Acknowledgements

This work was supported by grants from the "Ligue Nationale Française contre le Cancer" and the "SEITA".

References

[1] C.Leuchtenberger and R.Leuchtenberger, Proc. Ann. Assoc. Cancer Res. 9 (1968) 42.
[2] C.Leuchtenberger, M.Schumacher and T.Haldimann, Z. Präventivmed. 13 (1968) 130.
[3] N.Munsch and C.Frayssinet, Biochimie 53 (1971) 243.
[4] J.Chauveau, Y.Moulé and C.Rouiller, Exp. Cell Res. 11 (1956) 317.
[5] Y.Moulé, European J. Biochem. 17 (1970) 544.
[6] C.C.Widnell and J.R.Tata, Biochim. Biophys. Acta 123 (1966) 478.
[7] R.G.Roeder and W.J.Rutter, Proc. Natl. Acad. Sci. U.S. 65 (1970) 675.
[8] C.Kedinger, M.Gniazdowski, J.L.Mandel, F.Gissinger and P.Chambon, Biochem. Biophys. Res. Commun. 38 (1970) 165.
[9] G.G.Maul et T.H.Hamilton, Proc. Natl. Acad. Sci. U.S. 57 (1967) 1371.
[10] S.T.Jacob, E.M.Sajdel and H.N.Munro, Biochem. Biophys. Res. Commun. 38 (1970) 765.
[11] B.W.Kimes and D.R.Morris, Biochim. Biophys. Acta 228 (1971) 235.
[12] S.T.Jacob, E.M.Sajdel and H.N.Munro, Nature 225 (1970) 62.