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INFLUENCE OF ACTINOMYCIN D ON THE RNA METABOLISM
OF ANTIBODY-FORMING SPLEEN CELLS IN VITRO*

Biochemische Zeitschrift
Biochemical Journal
Vol 342, 1965, pages 387-391

1. Uridine-2-\textsuperscript{14}C in vitro is relatively rapidly incorporated into the RNA of spleen cells of rabbits. The highest amount of radioactivity is found in the 650 C fraction. Antibody-forming cells are especially active in RNA synthesis.

2. Actinomycin D strongly inhibits the RNA synthesis. While cells of normal rabbits are affected particularly in the 650 C fraction, in immunized animals the incorporation of uridine-2-\textsuperscript{14}C is inhibited in all fractions.

In an earlier work we were able to show that actinomycin D inhibits the in vitro synthesis of antibodies against alcohol dehydrogenase [1,2]. This finding suggested that a DNA-dependent RNA synthesis is necessary for the formation of antibodies. [See the list of abbreviations on page 2.] In the present article it is shown that actinomycin D inhibits RNA synthesis in the spleen cells. The experiments were carried out with cells from rabbits that had received an antigen injection before killing and 10-12 weeks after immunization with alcohol dehydrogenase.

**Materials**

Immunization with ADH, isolation of the spleen cells from rabbits, and determination of the antibody were carried out in accordance with [2,3].

**Acknowledgment**

Dr. S. Ochoa for his 60th birthday.

**Present address: Hygiene-Institut der Universität Freiburg.**
Short-Term Markings

6-7 g of spleen cells were pre-incubated in 120 ml of Eagle's medium for one hour at 37°C with and without actinomycin D (5 µg/ml), and then incubated for 20 minutes with uridine 2-14C (1 µCi/ml). An equal volume of ice-cold NaCl solution (0.14 M) was then added and the mixture centrifuged (10 minutes at 10,000 x g).

Isolation of RNA

The spleen cells obtained were homogenized in the Potter-Elvehjem apparatus with ten times their volume of ice-cold NaCl solution (0.14 M) with the addition of 1 mg/ml of bentonite (pretreated as in [4]). The mixture was then centrifuged (15 minutes at 800 x g), the cytoplasmatic liquid decanted, and the sediment washed twice more with NaCl solution.

Recovery of the Cytoplasmic RNA. — To the combined liquid was added up to 0.5% sodium deoxycholate and an equal volume of phenol (80%; pH 6.0). After 20 minutes' agitation at 4°C and centrifuging (10 minutes at 10,000 x g) the watery phase was pipetted off and the interphase again treated with ten times its volume of NaCl solution and a corresponding quantity of phenol.

The watery phases were mixed with alcohol up to 67%. This solution was kept overnight in the cold storage room. The RNA centrifuged off was dissolved in tris-HCl buffer (0.01 M; pH 7.4) with 0.001 M MgCl2, precipitated three times by means of potassium acetate (final concentration: 2 M) and ethanol (final concentration: 20%) and washed twice more with ethanol (95%).

The purified RNA was then dissolved in tris-HCl buffer (0.01 M; pH 7.4) with 0.001 M MgCl2.

Recovery of Nuclear RNA. — The nuclear sediment was homogenized with ten times its volume of NaCl solution (0.14 M) and then added to an equal volume of phenol (80%; pH 5.0). RNA fractions were then obtained from this by the method of Georgiev et al. [5] at 40°, 50°, and 65° C. The corresponding RNA's were isolated from the watery phases in each case as described above for the recovery of the cytoplasmic RNA.

The radioactivity was measured with the methane flow-
through counter tube. The specific radioactivity is given as
1 pm/μg (measured at 260 μm; thickness of layer 10 mm).

Gradient Centrifuging
This was done in accordance with the methods of Britten
et al. [6]. 8 OD of the isolated RNA, dissolved in 0.5 ml of
tris-HCl buffer (0.01 M; pH 7.4) with 0.001 M NaCl, was set
to a saccharose gradient (4.5 ml; 5-20% saccharose in 0.01 M
sodium acetate, pH 5.1; 0.1 M KCl; 0.001 M NaCl) and centri-
fuged in the preparation ultracentrifuge (Spinco L 55) in the
SW 39 rotor for five hours at 39,000 rpm. The centrifuge tube
was tapped and the contents caught by drops in separate tubes.

The content of the individual tubes was diluted with
1 ml of H2O, the extinction obtained at 260 μm, and an aliquot
transferred to aluminum dishes. The radioactivity was deter-
mained with the methane flow-through counter tube.

Other Methods
The base ratio was analysed by the method of Markham
et al. [7]. We thank Dr. U. Hagen, of the Radiological
Institute of the University of Freiburg in Breisgau, for de-
termining the sedimentation constants.

Preparations
We obtained ADH from C.F. Boehringer & Soehne GmbH,
Mannheim; Eagle’s medium from Grand Island Biological Co.
Grand Island, N.Y.; uridine-2-14C (30 mCi/mmol), uracil-2-14C
(20 mCi/mmol), and orotic acid 6-14C (8 mCi/mmol) from New
England Nuclear Corporation, Boston, Massachusetts. We have
the firm of Merck, Sharp & Dohme, Rahway, N.J., to thank for
the actinomycin D.

Results
1. Influence of actinomycin D on antibody synthesis.
Isolated spleen cells incubated in Eagle’s medium form
antibodies against alcohol dehydrogenase which can be shown
up by the enzymatic optical method [2]. This synthesis is
inhibited by actinomycin D even in a concentration of 5 μg/ml
(cf. Table 1).

Table 1
Synthesis of Antibodies Against ADH in Vitro Under the Influ-
ence of Actinomycin D. The cells from immunized animals were
incubated in Eagle’s medium for 20 hours. (For details see [2].)

<table>
<thead>
<tr>
<th>Addition of</th>
<th>Inhibition of the ADH Activity as Compared to Control Animals</th>
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<tbody>
<tr>
<td>5 μg/ml of actinomycin D</td>
<td>70%</td>
</tr>
<tr>
<td>0 μg/ml of actinomycin D</td>
<td>0%</td>
</tr>
</tbody>
</table>
2. Influence of actinomycin D on the RNA synthesis.

While the isolated cells hardly incorporate uracil-2-14C and orotic acid-6-14C into the RNA at all, uridine-2-14C is very rapidly taken up. For that reason uridine-2-14C was used for the short-term markings.

After 20 minutes' incubation with uridine-2-14C all the RNA fractions obtained by Georgiev's method [5] are marked. From Table 2 it may be seen that the RNA fractions from cells of immunized animals show a substantially higher specific activity than those from control animals. In both cases the highest specific activity is found in the 650°C fraction. This RNA approaches DNA in its base ratio. The greater part shows a Svedberg constant of $S_{20} = 8$.

Actinomycin D has a definite influence on the synthesis of the RNA isolated at 650°C. The incorporation of uridine-2-14C is reduced by more than half. (Cf. Table 2.) This result is also clear from Figures 1 and 2, where the sedimentation behavior of this RNA in a saccharose gradient (5-20%) is shown.

![Figure 1. Sedimentation diagram of RNA (650°C fraction) from spleen cells.](image-url)
But actinomycin D also shows a definite effect on the incorporation of uridine-2-$^{14}$C into the other RNA fractions (cf. Table 2). The effect on the fractions from immunized animals is substantially greater than on the controls.

**Discussion**

The inhibition of antibody synthesis in vitro by actinomycin D is accompanied by a definite reduction in the RNA synthesis. Both the nuclear RNA and the cytoplasmatic RNA are affected by this inhibition of synthesis. This finding is quite in harmony with the latest investigations of Smiley et al. [8].

From the above findings it may be concluded that in spleen cells from immunized animals the synthesis of antibodies is preceded by the formation of a DNA-dependent RNA.

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**Bibliography**