# *IN VIVO* EFFECT OF AFLATOXIN $B_1$ ON PROTEIN SYNTHESIS IN RAT LIVER

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## 1. Introduction

Aflatoxin $B_1$, a metabolite produced by some strains of *Aspergillus flavus*, is a very potent hepatocarcinogen. In short-term experiments, a single dose of toxin produces biochemical and morphological alterations in liver (see the review by Godblatt [1]).

It is currently admitted that aflatoxin $B_1$ impairs protein synthesis in liver, an effect that could be expected as a consequence of altered transcription in treated rats (see the review by Wogan and Pong [2]). However, the problem is far from clear. This is partly due to the differences in doses of aflatoxin $B_1$ administered by different authors; the reported use of high doses of aflatoxin, for example, led to acute toxic effects followed by a regenerative cellular process [3]. Moreover, *in vivo* experiments were performed at different levels (liver homogenate, slices, microsomes ...) and the findings are difficult to correlate. In some cases conflicting results were even reported for conditions apparently similar [3–5].

We have studied the mechanism of action of aflatoxin $B_1$ on liver protein synthesis taking into consideration the following questions: is the overall effect of the toxin solely a consequence of the alteration of RNA metabolism or, is there, in addition, a specific effect on polysomes and, if so, at what level of the translation?

The present paper deals with the time-course of aflatoxin $B_1$ on *in vivo* protein synthesis under conditions where no acute toxic lesion can be detected. The results are consistent with the hypothesis of a direct action of aflatoxin on the translational mechanism which takes place very early after dosing. Afterwards, the alteration of RNA synthesis may be considered to account for the impairment of protein synthesis in liver.

## 2. Materials and methods

Male Wistar rats (Commentry strain) weighing about 300 g and fed a semi-synthetic diet were fasted 15–16 hr before sacrifice.

Aflatoxin $B_1$ (Makor Inc., Israël) dissolved in propylene glycol was injected intraperitoneally at a dose of 1 mg/kg. Control rats received an equivalent volume of the toxin vehicle. The animals were killed by decapitation after the intervals of time indicated in the figures. In some cases, animals received an intraperitoneal injection of 12.5 $\mu$Ci of DL[$^{14}$C]-leucine 10 min before killing.

The soluble RNAase inhibitor from liver was prepared in 0.25 M sucrose in medium B according to Blobel and Potter [6]; it was used along each step of the cell fractionation.

The livers were quickly removed, rinsed and homogenized in 4 vol of 0.25 M sucrose made in medium B (50 mM Tris-HCl pH 7.6, 100 mM KCl, 5 mM Mg $(CH_3COO)_2$, 40 mM NaCl). The homogenate was filtered and centrifuged 10 min at 12,000 *g*; the postmitochondrial supernatant was carefully removed by aspiration.

Total RNP (polysomes + monomers + ribosomal subunits) were obtained by a previously described modification [7] of the original procedure published by Blobel and Potter [8]. Total RNP were overlayed on a 10–40% linear sucrose gradient in medium B and centrifuged 2 hr at 25,000 rpm (Spinco, rotor SW 25). The tube was fractionated in an ISCO model D

fractionator with an automatic recording of optical density. The ratio polysomes/total RNP was estimated by planimetry of the peaks of the record obtained after sucrose gradient centrifugation. Estimation of RNA on RNP fractions was determined by ultraviolet absorbance at 260 nm on 5% $HClO_4$ extracts performed at 70°.

The radioactivity of the proteins labelled *in vivo* was determined from 1 ml aliquots of liver homogenate. The tissue was precipitated and washed with ice-cold 5% $HClO_4$, extracted with cold alcohol and warm alcohol–ether (3:1). Proteins were dissolved in 0.5 N NaOH at 37°. Determination of proteins by the Lowry 's technic and measurement of radioactivity in a liquid scintillation spectrometer were made on the alkaline digest. The results were expressed in relative specific radioactivity (dpm per mg proteins/dpm of acid soluble pool).

## 3. Results and discussion

The sensitivity of the rats to aflatoxin $B_1$ depends primarily on their strain. With the strain used in these experiments, we found that a dose of 1 mg/kg of aflatoxin $B_1$ produces no acute hepatoxic lesion such as periportal necrosis or biliary proliferation.

A single injection of aflatoxin $B_1$ strongly inhibits *in vivo* protein synthesis in liver (fig. 1, curve A). The time-course of inhibition reveals a biphasic response: a marked effect which reaches a peak 2 hr after dosing, and then declines up to 7 hr. Thereafter, the inhibition progressively increases to a level of about 85%. No attempt was made to evaluate the effect beyond 48 hr; these experiments were specifically aimed at studying the early effects of aflatoxin administration. Such a pattern of inhibition reveals the complex mechanism of the toxin's effect.

It must be pointed out that aflatoxin $B_1$ does not modify the uptake of amino acids in the liver cell; the results we have obtained confirm those previously reported [4, 5]. However, in order to exclude any possible individual variability in the acid soluble pool, the present results have been expressed in terms of relative specific radioactivity.

Inhibition of protein synthesis is directly related to quantitative and qualitative changes in polysomes. It is known that aflatoxin $B_1$ dramatically impairs

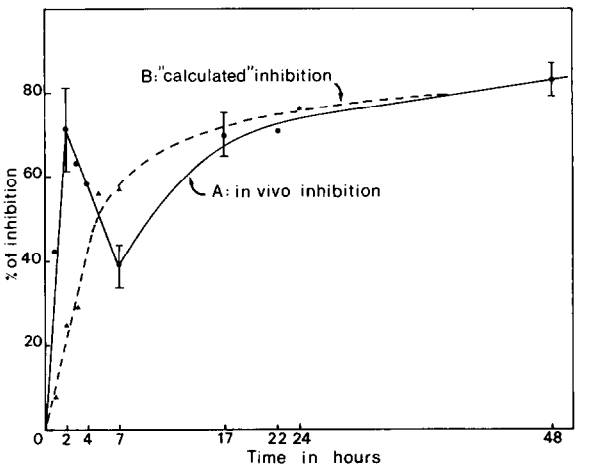


Fig. 1. Time-course inhibition of protein synthesis by aflatoxin $B_1$ in rat liver. Rats were killed after administration of aflatoxin $B_1$ (1 mg/kg) at time intervals as indicated on the graph; 10 min before killing, they received an intraperitoneal injection of 12.5 μCi of DL[$^{14}$C]leucine. Curve A ●—●—●: Percentage of *in vivo* inhibition in total liver proteins. Curve B ▲- - -▲- - -▲: Protein synthesis inhibition calculated from the effective loss of active polysomes (see text and table 1). For each interval of time, the number of experiments varied between 3 and 9. The vertical bars correspond to the standard error $s_m \sqrt{\Sigma(x-\bar{x})^2/N(N-1)}$.

Table 1
Quantification of the inhibition of protein synthesis due to altered transcription in aflatoxin $B_1$-treated rats.

| Time after aflatoxin injection (hr) | Percent of polysome degradation | Percent of total RNP (estimated in RNA) | Percent of protein synthesis inhibition |
|---|---|---|---|
| | A | B | C |
| Control | (0) | (100) | (0) |
| 0.5 | 7 | 100 | 7 |
| 1 | 8 | 100 | 8 |
| 2 | 14 | 87 | 25 |
| 3 | 26 | 96 | 29 |
| 5 | 39 | 72 | 56 |
| 7 | 39 | 70 | 57 |
| 15 | 62 | – | – |
| 24 | 55 | 53 | 76 |

Percent of protein synthesis inhibition (C) calculated on the basis of polysomes degradation (A) and loss in total RNP (B) was expressed as follows: $C = 100 - B(1-A/100)$.

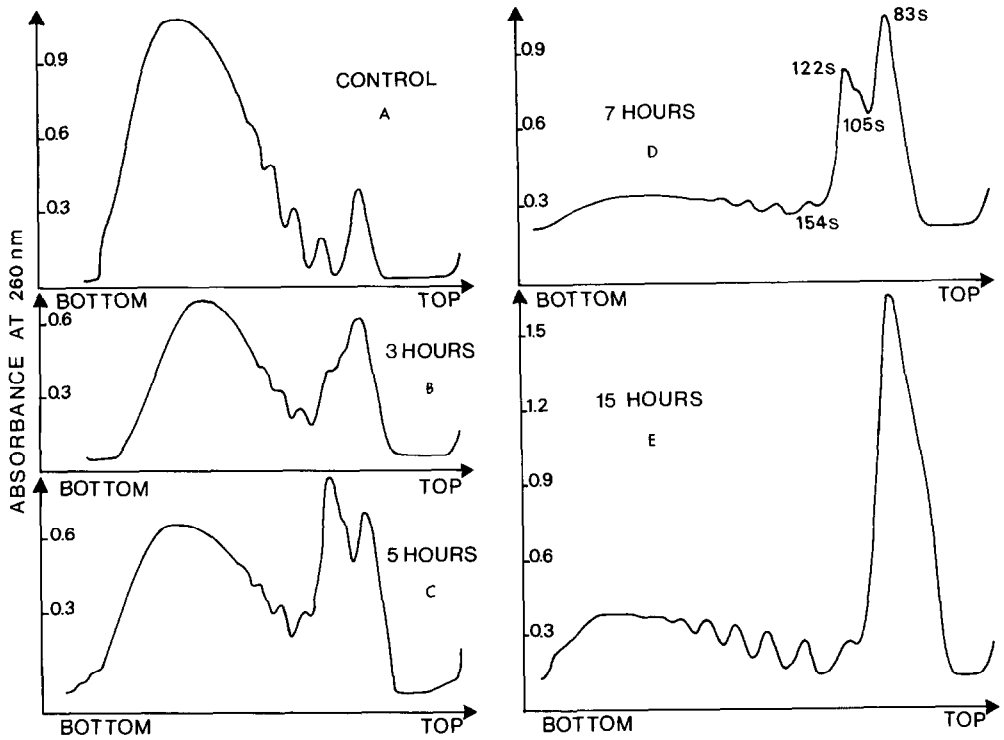


Fig. 2. Sucrose density gradient pattern of total RNP in control and aflatoxin $B_1$-treated rats. Experimental conditions were identical to those indicated in fig. 1. Polysomes were isolated as described in Materials and methods and centrifuged for 2 hr at 25,000 rpm in a 10–40% linear sucrose gradient in medium B. A: control; B, C, D, E: 3 hr, 5 hr, 7 hr and 15 hr, respectively after *in vivo* aflatoxin $B_1$ dosing.

RNA metabolism in liver (references in [1]). In fact, the amount of total RNP progressively decreases after toxin injection reaching a value of 50% as compared to that of controls 24 hr after dosing (table 1). Moreover, in normal liver total RNP consist primarily of functional polysomes; "light particles" (dimers + monomers + subunits) are simultaneously observed but in much lower quantities (fig. 2A). Aflatoxin $B_1$ induces marked changes in the pattern of sedimentation of total RNP: polysomes become disaggregated leading to a higher proportion of "light particles" (fig. 2, B to E). Similar results were previously reported [5, 9, 10].

The quantification of this effect was estimated by the ratio between the area of the peaks corresponding respectively to polysomes and "light fractions" (table 1). Disaggregation of polysomes starts 3–4 hr after aflatoxin administration then progressively increases, reaching a maximum of 50–60% (fig. 3).

Calculation of the amount of polysomes available for each interval of time after toxin dosing was made from values corresponding to the amount of total RNP and the percentage of polysomes in the RNP population (table 1). This allows us to obtain a curve representing the inhibition of protein synthesis which may be expected on the basis of the residual amount of functional aggregates (fig. 1, curve B).

It can be seen that the calculated curve coincides fairly well with that representing *in vivo* inhibition of protein synthesis for intervals of time beyond 7 hr. Thus, impairment of transcription by aflatoxin $B_1$ which leads to inhibition of both ribosomal RNA synthesis [11] and messenger RNA induction (references in [2]) might account for alteration in liver protein metabolism beyond 7 hr. However, the defect in RNA production cannot explain the short term inhibition occurring within the first 7 hr after treatment.

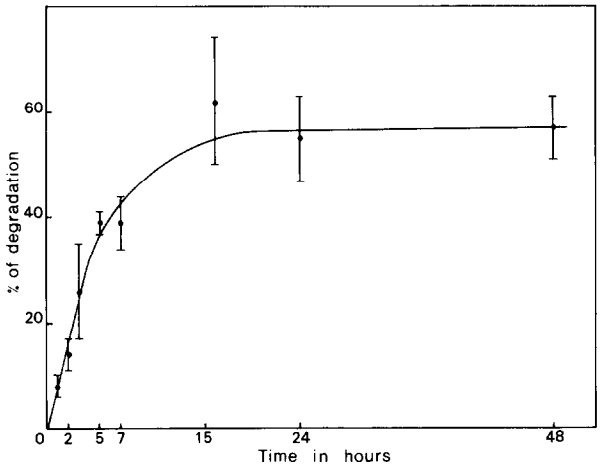


Fig. 3. Time-course degradation of polysomes in aflatoxin $B_1$-treated rats. The results were estimated from the areas of the peaks of sedimentation gradient patterns shown in fig. 2; they were expressed as percentage of inhibition with regard to the control value. For each interval of time, the number of experiments varied between 4 and 10. The standard error was calculated as indicated in the legend of fig. 1.

A direct action of aflatoxin $B_1$ on the translational process (apart from any effect via transcription) might account for the early inhibition in protein synthesis. Morphological changes in structural conformation of polysomes such as appearance of helical polysomes detected in the cytoplasm within 60 min after toxin injection [12] would lend support to such a hypothesis. Moreover, preliminary experiments have shown that *in vivo* synthetic activity of polysomes in treated rats is significantly lowered with regard to the controls at 2 hr time intervals (results to be published). Experiments are now in progress to define more precisely the mechanism of the early action of aflatoxin $B_1$ on the translational process in liver.

In conclusion, *in vivo* effect of aflatoxin $B_1$ on protein synthesis in rat liver could be sequentially divided as follows: up to 7 hr, the drug would act directly on the functional polysomes leading to a marked inhibition which culminates at 2 hr. Beyond 7 hr, inhibition of protein synthesis is primarily a consequence of the impairment of the transcriptional process induced by the toxin.

## Acknowledgements

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