Biochemical Pharmacology, Vol. 18, pp 2357–2361. Pergamon Press. 1969. Printed in Great Britain

TIME COURSE OF ALTERATIONS OF RAT LIVER POLYSOME PROFILES INDUCED BY AFLATOXIN B_1*

RAYMOND S. PONG and GERALD N. WOGAN

Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Mass. 02139, U.S.A.

(*Received* 16 *January* 1969; *accepted* 11 *April* 1969)

Abstract—Aflatoxin B_1 administered intraperitoneally (i.p.) as a single dose to rats at a dose level of 3 mg/kg (the LD_{50}) caused marked, but reversible, disaggregation of liver polysomes. Although disaggregation was not evident within 0·5 hr, it was extensive at 3 hr and persisted through 36 hr after dosing. Partial reaggregation began at 36 hr, and the process was essentially complete by 5 days after dosing.

AFLATOXIN B_1, a toxic and carcinogenic metabolite of some strains of *Aspergillus flavus*, is a potent inhibitor of liver nuclear RNA synthesis when administered acutely to rats.[1,2] Under similar conditions, the toxin also inhibits induction of soluble and microsomal enzymes.[3,4] Incorporation *in vitro* of amino acids into protein of liver slices and cell-free preparations is suppressed in the presence of the toxin.[5,6] A recent report associated these effects on protein synthesis with alterations in ribosomal aggregation.[7]

Because many of these effects appear to be at least partially reversible, we have investigated the time course of alterations in the rat liver polysome profile induced by a single dose of the toxin.

MATERIALS AND METHODS

Aflatoxin B_1 used in this study was produced by submerged culture of *Aspergillus flavus* (ATCC 15517) as described by Mateles and Adye.[8] The aflatoxin B_1 isolated was more than 99·5 per cent pure, as demonstrated by thin-layer chromatography on silica gel plates and molar extinction coefficient ($2{\cdot}2 \times 10^4$) at 363 mμ.[9]

Male Fischer rats weighing about 100 g were injected i.p. with 3 mg/kg (LD_{50}) of aflatoxin B_1 dissolved in 0·05 ml of spectral grade dimethylsulfoxide. They were sacrificed by decapitation at 9 a.m. at intervals of 0·5 hr to 5 days after dosing. Polysome profiles were prepared from postmitochondrial fractions of liver homogenate, as described by Drysdale and Munro,[10] using rabbit antiserum to horse ferritin (Calbiochem, Los Angeles, Calif.) to remove ferritin. In each experiment, a constant volume (0·1 ml) of the postmitochondrial fraction was layered onto a linear 10–40 per cent (w/v) sucrose gradient. The gradients were centrifuged at 4°, 35,000 rpm in a Spinco SW 50 rotor for 50 min in a Spinco L ultracentrifuge and were displaced up-

*Preliminary accounts of this work were presented at the 1968 Annual Meeting of the Federation of American Societies for Experimental Biology. Supported by Contract No. PH 43-62-468 from the National Cancer Institute, NIH. Contribution No. 1421 from the Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Mass.

wards into a 0·2-cm flow cell. The optical density of the respective gradients was continuously recorded at 260 mμ in a Gilford spectrophotometer.

At each reported time interval, profiles from three saline-treated, two dimethylsulfoxide-treated, and four aflatoxin-treated rats were individually recorded. Profiles reported in Figs. 1 and 2 represent actual tracings of typical results.

Quantitation of observed changes in the polysome profiles was accomplished through determination, by planimetry, of the areas under the monomer and dimer peaks and the total areas of the respective profiles. The areas (monomer plus dimer) were calculated as percentages of the total areas.

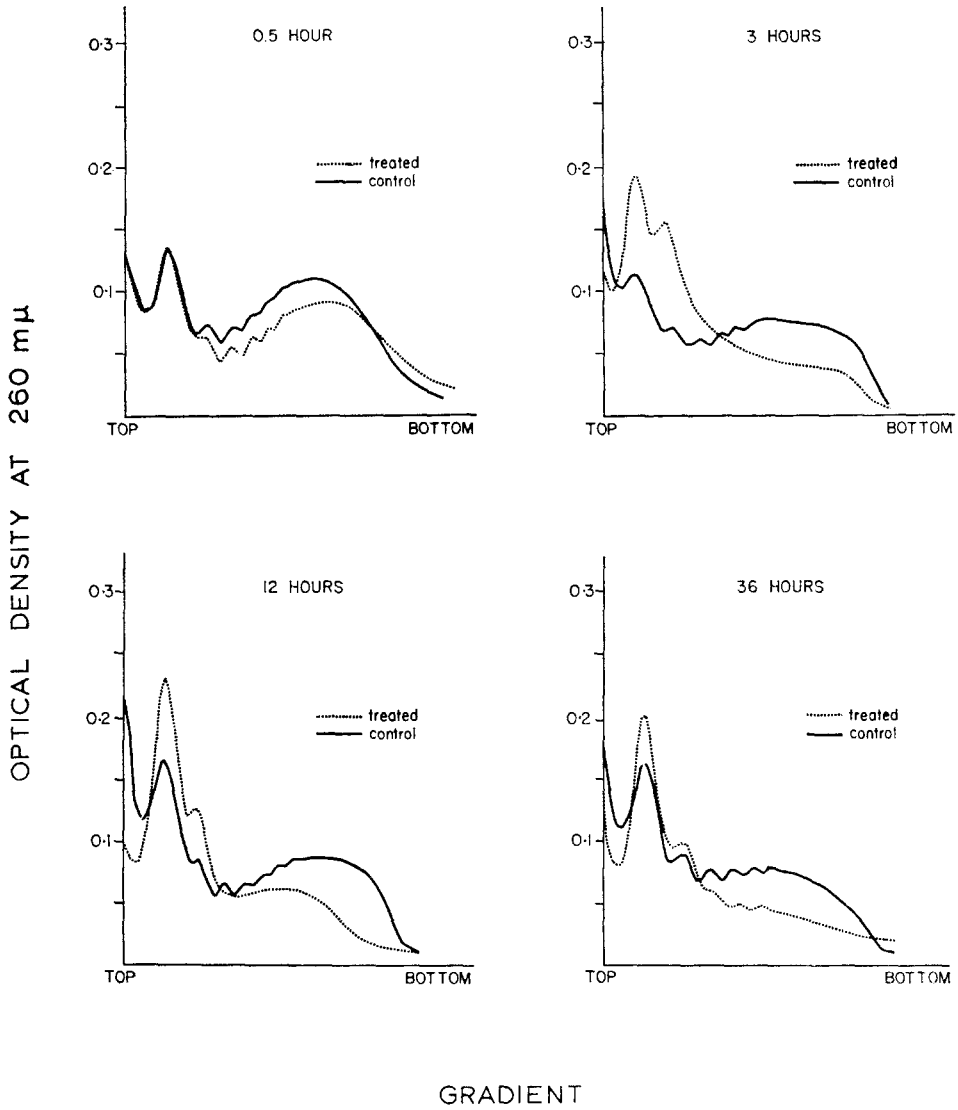

FIG. 1. Rat liver polysome profiles 0·5, 3, 12 and 36 hr after a single i.p. dose of 3 mg/kg of aflatoxin B_1.

RESULTS

Polysome profiles are recorded in Figs. 1 and 2, and a quantitative expression of the principal observed changes is presented in Table 1. Dimethylsulfoxide, the vehicle for the toxin, was found in preliminary studies not to alter the polysome profile when compared to saline. The control profile shown at each time interval in Figs. 1 and 2 therefore represents the dimethylsulfoxide control done at that time. The control profiles in each instance consist of at least five oligosome peaks and one large polysome peak and agrees well with previously published profiles from untreated rats.

In animals treated with aflatoxin B_1 and killed 0·5 hr after dosing, the polysome profile was not significantly different from that of control animals (Fig. 1). The monomer

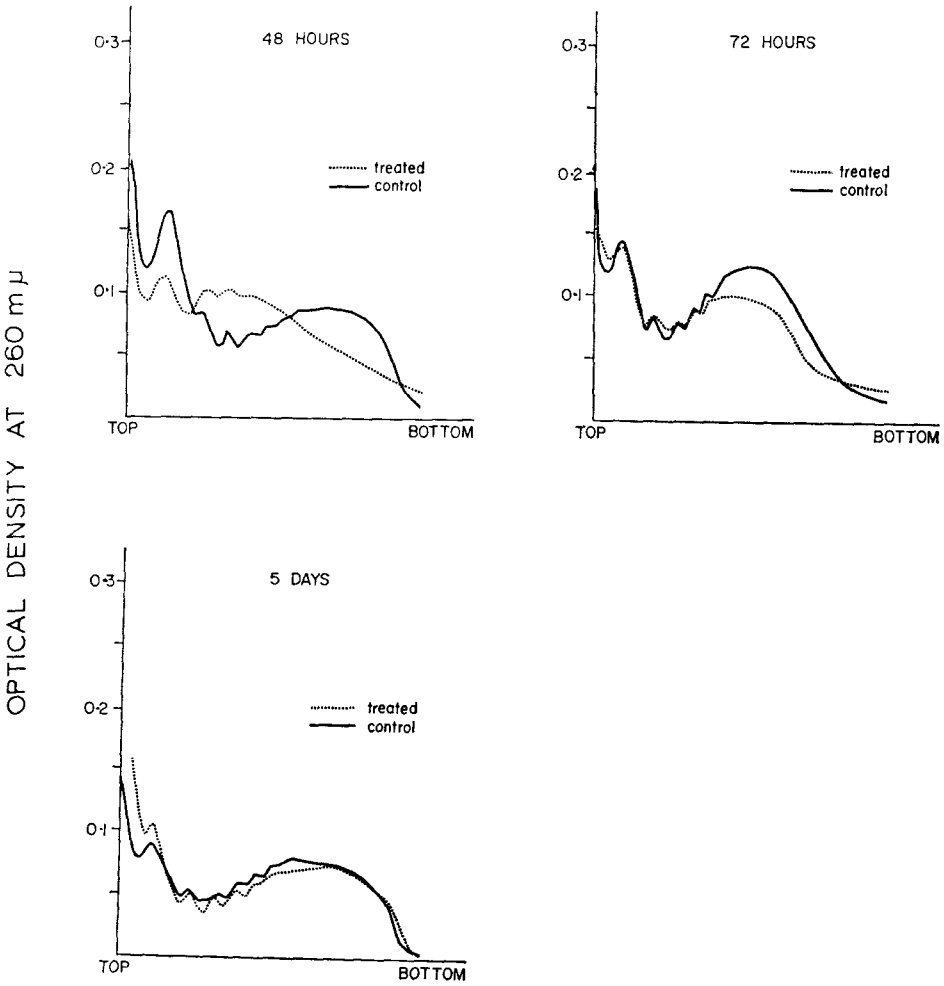

FIG. 2. Rat liver polysome profiles 48 and 72 hr and 5 days after a single i.p. dose of 3 mg/kg of aflatoxin B_1.

plus dimer areas were similar in the treated and in the control, comprising approximately 30 per cent of the total profile area (Table 1). However, 3 hr after treatment, the polysome segment of the profile was decreased, with corresponding increases in the monomer–dimer areas, amounting to an increase, of nearly 50 per cent over the control value. The polysome area was still decreased and the monomer–dimer fraction increased to approximately the same extent (46 per cent) at 12 hr after dosing with the toxin. After 36 hr, reaggregation had begun, with the profile showing an increase of only 23 per cent in the monomer–dimer areas as compared to control.

TABLE 1. ALTERATIONS IN POLYSOME PROFILES CAUSED BY AFLATOXIN B_1

Time after aflatoxin B_1 injection (hr)	Monomer + dimer areas (% of total profile)*		Treated control
	Dimethylsulfoxide control	Aflatoxin B_1-treated	
0·5	29·9	30·2	1·01
3	32·1 (30·5–33·6)	48·2 (45·5–53·5)	1·48 (1·35–1·75)
12	30·9 (30·0–37·7)	47·3 (42·9–57·4)	1·46 (1·43–1·52)
36	36·2 (30·7–41·7)	42·5 (36·0–53·5)	1·23 (1·17–1·28)
48	37·6	35·0	0·93
72	26·1	28·7	1·10
120	26·1	24·0	0·92

*Mean (and range) of values obtained from one to three individual profiles.

Figure 2 shows profiles from rats killed 48, 72 and 120 hr after dosing. The reaggregation that had begun earlier continued through these time intervals as indicated by progressive increases in the polysome area of the profile and return of the monomer–dimer areas to control values (Table 1). Reaggregation was essentially complete by 72 hr, and within 5 days profiles from treated and control rats were essentially identical.

DISCUSSION

Results presented here demonstrate that i.p. administration of aflatoxin B_1 to rats at a dose level of 3 mg/kg causes marked disaggregation of liver polysomes, which is clearly evident within 3 hr and persists until 36 hr after toxin administration. Monomer and dimer areas of the polysome profile return to control levels after 72 hr, but polysome reaggregation is not entirely complete until 5 days after treatment.

It can be seen in Figs. 1 and 2 that increases in oligosome areas are not equivalent to decreases in polysome areas during the interval from 12 to 72 hr after treatment. The disproportionate loss of polysomes is possibly accounted for by loss of cellular (principally ribosomal) RNA in rats treated similarly, as described elsewhere.[11] These earlier studies showed that there was a loss of RNA of approximately 40 per cent within 12 hr after dosing, and this loss persisted beyond 72 hr.

These observations on polysome profiles correlate in general with electron microscopic observations by Butler[12] and Svoboda *et al.*[13] that rat liver ribosomes dissociate from the endoplasmic reticulum after aflatoxin B_1 treatment. The polysomal disaggregation reported here also resembles qualitatively the similar response caused by actinomycin. It has been reported[14] that this compound, in doses of 0·6 to 3·0 mg/kg

to rats, causes almost complete disaggregation of liver polysomes to monomers and dimers within a period of 4 hr. Previous evidence[3,4] indicates that aflatoxin B_1 and actinomycin inhibit messenger RNA synthesis with similar characteristics, and the present findings further suggest that the two inhibitors act through similar mechanisms.

A cytoplasmic effect of the toxin is also suggested by experiments *in vitro*, which demonstrate an inhibition of protein synthesis by the compound.[5,6] However, the time course of the effects of aflatoxin B_1 on rat liver protein synthesis *in vivo*[15] does not correlate with the findings reported here. ^{14}C-leucine incorporation *in vivo* is only transiently inhibited by aflatoxin B_1 and, by 12 hr after dosing, the incorporation of labeled amino acid has returned to control levels. It is difficult to explain unchanged protein synthesis *in vivo* at a time (12 hr after treatment) when maximal disaggregation of polysomes is evident. This apparent ambiguity may result from alterations induced by the toxin in the hepatocyte endoplasmic reticulum. Such effects are suggested by the previous findings that the activity of zoxazolamine hydroxylase, a microsomal enzyme, induced by 3, 4-benzpyrene decays more rapidly in aflatoxin-treated rats than in controls.[4] It is probable that decay of enzyme activity under these conditions is associated with alterations in the physical integrity of the membranes of the endoplasmic reticulum.

The additional possibility that the observed effects on leucine incorporation may be due to alterations in amino acid pool size induced by the toxin has not been evaluated experimentally.

Acknowledgements—Drs. J. Berger and A. Brossi kindly provided us with an aflatoxin concentrate prepared by Mr. B. Tabenkin at Hoffman-LaRoche, Inc., Nutley, N.J.

REFERENCES

1. C. Lafarge, C. Fraysinnet and A. M. De Recondo, *Bull. Soc. Chim. biol.* **47**, 1724 (1965).
2. M. B. Sporn, C. W. Dingman, H. L. Phelps and G. N. Wogan, *Science, N.Y.* **151**, 1539 (1966).
3. G. N. Wogan and M. A. Friedman *Archs Biochem. Biophys.* **128**, 509 (1968).
4. R. S. Pong and G. N. Wogan, *Fedn Proc.* **25** (2), 662 (1966).
5. R. H. Smith, *Biochem. J.* **88**, 50 (1963).
6. J. I. Clifford and K. R. Rees, *Biochem. J.* **102**, 65 (1967).
7. S. Villa-Trevino and D. D. Leaver, *Biochem. J.* **109**, 87 (1968).
8. R. I. Mateles and J. C. Adye, *Appl. Microbiol.* **13**, 208 (1956).
9. T. Asao, G. Büchi, M. M. Abdel-Kader, S. B. Chang, E. L. Wick and G. N. Wogan, *J. Am. chem. Soc.* **87**, 882 (1965).
10. J. W. Drysdale and H. N. Munro, *Biochim. biophys. Acta* **138**, 616 (1967).
11. M. A. Friedman and G. N. Wogan, *Fedn Proc.* **26**, 358 (1967).
12. W. H. Butler, *Am J. Path.* **49**, 113 (1966).
13. D. Svoboda, H. Grady and J. Higginson, *Am. J. Path.* **49**, 1023 (1966).
14. T. Staehelin, F. O. Wettstein and H. Noll, *Science, N.Y.* **140**, 180 (1963).
15. R. C. Shank and G. N. Wogan, *Toxic. appl. Pharmac.* **9**, 468 (1966).