Time Course and Dose-Response Characteristics of Aflatoxin B₁ Effects on Rat Liver RNA Polymerase and Ultrastructure

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SUMMARY

Treatment of 100-g male Fischer rats with an i.p. dose of 1 mg/kg of aflatoxin B₁ causes a rapid and marked inhibition of the activity of liver DNA-dependent RNA polymerase. Over a period of 36 hr after dosing, ultrastructural alterations of the liver cell nucleolus generally correlate with the inhibition of enzyme activity. Mg²⁺-activated and Mn²⁺-(NH₄)₂SO₄-activated RNA polymerase activities, 15 min after dosing, are maximally inhibited by about 60%; this inhibition continues to 12 hr. By 36 hr, enzyme activities have returned to pretreatment levels. Hepatocyte nucleoli 15 min after treatment show decreased prominence of nucleolonema, and nucleolar microsegregation is observed. Macrosegregation of granular and fibrillar nucleolar components (nucleolar capping) is demonstrated within 1 hr after treatment, and the segregation persists up to 12 hr. By 36 hr, reintegration of nucleolar components is observed.

Inhibition of RNA polymerase activity is directly related to aflatoxin B₁ dose, with a correlation coefficient of −0.89 for the Mg²⁺-activated system and −0.97 for the Mn²⁺-(NH₄)₂SO₄-activated system. Nucleolar disorganization is observable at 0.2 mg/kg aflatoxin B₁ but is not demonstrable at 0.1 mg/kg, a dose which is capable of a 5% inhibition of both types of enzyme activity. A decrease in nuclear RNA/DNA ratio is observed under conditions where RNA polymerase activity is decreased.

INTRODUCTION

Morphological and biochemical lesions caused by acute administration of aflatoxin B₁ to animals appear almost exclusively in liver and persist for variable lengths of time, depending upon dose levels and other parameters. Nucleolar segregation together with decreased nucleolar size in rat hepatocytes represent consistently observed ultrastructural alterations associated with acute toxicity of the compound (2, 4, 22). Biochemical effects of the toxin observed under similar conditions include inhibition of liver cell nuclear RNA synthesis (5, 8, 20), a secondary consequence of inhibition of RNA polymerase activity (6, 9, 10).

The present experiments were undertaken to provide further information on several aspects of hepatocyte nuclear responses. Specifically, earlier studies have, for the most part, involved observations made at one or a few fixed time intervals following toxin treatment. Also, they have often been made with doses of aflatoxin that are either lethal or strongly cytotoxic. Therefore, the possible importance of nonspecific responses to cellular necrosis and also the contribution of cytotoxicity to persistence or reversibility of observed effects in intact rats have not previously been systematically investigated.

Furthermore, previous studies on RNA polymerase inhibition have been done with only the enzyme assay system activated by magnesium ions and ammonium sulfate. Widnell and Tata (27) have reported 2 types of DNA-dependent RNA polymerase activities in rat liver nuclei, one activated by magnesium, the other by manganese-ammonium sulfate. These authors inferred that activation under the 2 conditions was associated with synthesis of ribosomal RNA or DNA-like RNA, respectively. Therefore, it was of considerable interest to investigate whether suppression by aflatoxin B₁ was equally effective under both conditions of enzyme activation. Available biochemical and morphological evidence suggests that inhibition of RNA polymerase activity and hepatic nucleolar segregation, resulting from aflatoxin treatment, are functionally associated. However, it has not been shown whether the 2 responses are temporally related, since no previous experiments have involved ultrastructural studies during the earliest phases of the biochemical response, or simultaneous ultrastructural and biochemical studies in the same animals.

Therefore, we have investigated the time course of effects in rats of a nonlethal dose (1 mg/kg) of aflatoxin B₁ on both RNA polymerase activities and on hepatocyte nucleolar ultrastructure. In addition, we have studied the dose-response characteristics of RNA polymerase inhibition and nucleolar segregation induced by the toxin.

MATERIALS AND METHODS

Aflatoxin B₁ used in these experiments was produced and purified by methods previously described (29). The compound was more than 99.5% pure as demonstrated by thin-layer chromatography on silica gel plates and molar
extinction coefficient \((2.2 \times 10^4)\) at 363 nm \((1)\). It was administered to animals as a solution in spectral grade dimethyl sulfoxide (Burdick and Jackson Laboratories, Muskegon, Mich.).

ATP, CTP, GTP, and UTP were obtained from Sigma Chemical Company (St. Louis, Mo.). ATP-8-\(^{14}\)C was obtained from Schwarz BioResearch, Inc. (Orangeburg, N. Y.).

Animals used were male Fischer rats, weighing about 100 g each, obtained from Charles River Breeding Laboratories (North Wilmington, Mass.). Each animal was given intraperitoneal injections of the prescribed dose of aflatoxin B1 dissolved in 0.05 ml DMSO. In all instances the animals were sacrificed by decapitation at 9 a.m., and samples for RNA polymerase assay and electron microscopy were taken from all 4 lobes of the same liver. Liver nuclei were isolated, as described by Dingman and Sporn \((7)\), with a Spincov SW25 rotor. Nuclear preparations were considered to be of acceptable purity for enzyme activity determinations only when phase microscopy revealed the nuclei to be round, with visible nucleoli and smooth nuclear membrane, and when the nuclear RNA/DNA ratio was less than 0.25.

Tissue samples for electron microscopy were fixed in glutaraldehyde \((19)\) and postfixed in 1% OsO\(_4\) in phosphate buffer \((15)\). They were then dehydrated in a graded series of alcohols and finally embedded in Epon 812 \((13)\). Silver sections, cut with a diamond knife on an LKB 1 microtome, double-stained with uranyl acetate \((25)\) and lead citrate \((18)\), were examined with a Philips EM 200 electron microscope at magnifications varying from \(\times 9,000\) to \(\times 61,000\). Ultrastructural aspects of this report are based on study and analysis of about 600 micrographs, and effects reported are representative of changes observed under various experimental conditions.

In the experiment dealing with the time course of effects of 1 mg/kg aflatoxin B1 on DNA-dependent RNA polymerase activity, rats were sacrificed in groups of 4 at intervals from 5 min to 36 hr after dosing. The activities of RNA polymerase activated by magnesium and by manganese sulfate were determined in liver nuclei according to the procedures of Widnell and Tata \((27)\). Briefly summarized, the incubation mixture for both assays contained the following common components: GTP, CTP, UTP, AlP, and an aliquot of nuclear suspension isolated from 8 g pooled liver from 4 rats. For the Mg\(^{++}\)-dependent activity, the following components were added: Tris buffer, pH 8.5; MgCl\(_2\); cysteine; and NaF. The medium for assay of Mn\(^{++}\)-(NH\(_4\))\(_2\)SO\(_4\)-dependent activity contained, in addition to common components: Tris buffer, pH 7.5; MnCl\(_2\), and saturated (NH\(_4\))\(_2\)SO\(_4\), pH 7.5. In the Mg\(^{++}\)-activated assay, ATP-8-\(^{14}\)C was added to the medium which was then incubated at 37° for 15 min. The Mn\(^{++}\)-(NH\(_4\))\(_2\)SO\(_4\)-activated system was incubated in the presence of ATP-8-\(^{14}\)C for 45 min at 37° after the medium had been preincubated for 15 min at the same temperature. The assays were stopped by addition of perchloric acid; RNA was extracted and precipitated by the method of Widnell and Tata \((26)\), and radioactivity measurements were made in a Packard Tri-Carb liquid scintillation spectrometer. Nuclear RNA and DNA contents were determined by the method of Munro and Fleck \((16)\).

In a dose-response experiment, the same procedures were used to determine the effects of 0.5, 0.2, 0.1, or 0.05 mg/kg aflatoxin B1 on the 2 types of RNA polymerase activity, except that rats were sacrificed (in groups of 4) 15 min after dosing.

RESULTS

Chart 1 demonstrates the lack of effect of a single dose of 0.05 ml DMSO, the aflatoxin vehicle, on RNA polymerase activity. Neither the Mg\(^{++}\)-activated nor the Mn\(^{++}\)-(NH\(_4\))\(_2\)SO\(_4\)-activated system was significantly affected by the solvent. Throughout the time period studied, observed alterations in enzyme activity were randomly distributed and did not exceed \(\pm 10\%\) of the NaCl control values. Similarly, hepatocyte nucleolar ultrastructure was not altered by the solvent, as shown in Fig. 1. Furthermore, the nuclear RNA/DNA ratios (Table 1) from animals treated with DMSO were comparable to NaCl-treated controls in all instances.

Chart 2 records the effects of aflatoxin B1 administered at a dose level of 1 mg/kg body weight, on the activity of DNA-dependent RNA polymerase. The activity of the Mg\(^{++}\)-activated system was depressed to 60% of the control value within 5 min after treatment and to 36% of control value within 15 min. Maximal suppression persisted through 12 hr, but by 36 hr after treatment enzyme activity had returned to control levels. RNA polymerase activated by Mn\(^{++}\)-(NH\(_4\))\(_2\)SO\(_4\) was similarly inhibited by aflatoxin B1. The enzyme activity was slightly inhibited 5 min after dosing, and there was rapid depression of activity between 5 and 15 min. By 15 min, the inhibition was virtually maximal and by 12 hr, the enzyme activity was 25% of the DMSO control. At 36 hr after treatment the RNA polymerase activity had returned to the control value.

Table 1 records nuclear RNA/DNA ratios at intervals following injection of aflatoxin B1. A loss of nuclear RNA was observed within 15 min after dosing \((0.169\text{ compared to } 0.237 \text{ for DMSO control, and } 0.225 \text{ for the saline control})\). The loss of nuclear RNA continued up to 12 hr after treatment when it was maximal \((\text{ratio of } 0.146)\). The RNA/DNA ratio returned to control levels by 36 hr at which time RNA polymerase activity was also comparable to the control level.

The electron microscopic findings following administration of 1 mg/kg aflatoxin B1 showed nucleolar alterations that correlate well with the time course of RNA polymerase inhibition by the same dose of the toxin. The nucleolus, 5 min after dosing, showed no apparent structural alterations from that of the DMSO control (Fig. 1), which consisted of a branching and anastomosing coarse strand of integrated granular \((g)\) and fibrillar \((f)\) component in an amorphous matrix with surrounding nucleolus-associated chromatin \((Ch)\) and perichromatinic granules \((p)\). Fig. 2 shows a rat liver nucleolus 15 min after treatment. The nucleolonema was not

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\(^3\)The abbreviation used is: DMSO, dimethyl sulfoxide.
Table 1

<table>
<thead>
<tr>
<th>Time after treatment</th>
<th>RNA/DNA</th>
<th>NaCl control</th>
<th>DMSO control</th>
<th>Aflatoxin-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 min</td>
<td></td>
<td>0.232 ± 0.01</td>
<td>0.228 ± 0.01</td>
<td>0.235 ± 0.01</td>
</tr>
<tr>
<td>15 min</td>
<td></td>
<td>0.225 ± 0.01</td>
<td>0.237 ± 0.01</td>
<td>0.169 ± 0.01</td>
</tr>
<tr>
<td>1 hr</td>
<td></td>
<td>0.242 ± 0.01</td>
<td>0.248 ± 0.01</td>
<td>0.162 ± 0.01</td>
</tr>
<tr>
<td>12 hr</td>
<td></td>
<td>0.225 ± 0.01</td>
<td>0.225 ± 0.01</td>
<td>0.146 ± 0.01</td>
</tr>
<tr>
<td>36 hr</td>
<td></td>
<td>0.215 ± 0.01</td>
<td>0.224 ± 0.01</td>
<td>0.218 ± 0.01</td>
</tr>
</tbody>
</table>

Prominent. In addition, there was a microsegregation of granular (g) and fibrillar (f) components. These nucleolar alterations induced by aflatoxin B₁ occurred simultaneously with maximal inhibition of DNA-dependent RNA polymerase activity by the toxin (Chart 2). Within 1 hr after dosing with aflatoxin B₁, nucleolar capping or macrosegregation was observed. A fibrillar area (f) was found between 2 granular zones (g) (Fig. 3). The nucleolus was decreased in size, and nucleolus-associated chromatin (Ch) was observed. Macrosegregation of nucleolar components was still present at 12 hr after dosing, but by 36 hr, nucleolar capping was not demonstrable and the fibrillar and granular components were well integrated. The nucleolonema was prominent.

As shown in Chart 2, inhibition of DNA-dependent RNA polymerase activity was essentially maximal 15 min after
dosing with aflatoxin B₁. Thus, in the dose-response experiment, the treated rats were sacrificed 15 min after injection with doses of aflatoxin B₁ over the range of 0.05 to 0.5 mg/kg. Data presented in Chart 3 indicate that aflatoxin dose is linearly related to inhibition of Mg⁺⁺-activated RNA polymerase. The correlation coefficient (−0.89) indicates a strong relationship between dose and response. From the linear regression line, it can be seen that a dose level of 0.08 mg aflatoxin B₁/kg body weight represents the maximal dose without measureable effect.

Comparable data for Mn⁺⁺-(NH₄)₂SO₄-activated RNA polymerase are presented in Chart 4, in which it can be seen that a linear relationship between aflatoxin dose and enzyme inhibition exists. The correlation coefficient (−0.97) again indicates a strong correlation between the 2 parameters. In this instance, the dose at which aflatoxin B₁ caused no apparent effect on enzyme activity is 0.065 mg/kg.

In view of results obtained in the above dose-response experiments, it was important to determine whether the lack of inhibition observed in the experiment with 0.05 mg/kg of toxin treatment for 15 min was the result of rapid reversal of inhibition within that time. An experiment was therefore performed in which rats were killed 5 min after dosing with 0.05 mg/kg aflatoxin B₁. There was no observable inhibition of enzyme activity or loss of nuclear RNA under these conditions.

Table 2 presents nuclear RNA/DNA ratios 15 min after treatment with various doses of aflatoxin B₁. Loss of nuclear RNA, as indicated by the RNA/DNA ratio, was observed at dose levels between 0.5 and 0.1 mg/kg, the extent of loss being directly related to dose level. No significant decrease in the RNA/DNA ratio was observed at a dose of 0.05 mg/kg.

Liver nucleolar ultrastructure from animals used in the dose-response experiment is presented in Fig. 4. At a dose level of 0.2 mg/kg of aflatoxin B₁ (at which enzyme activities were inhibited by 20%), the nucleolus showed disorganization of its components. The granular and the fibrillar components were not well integrated and the nucleolonemas were not as well organized as the control. A nucleolus from an animal treated with 0.1 mg/kg of aflatoxin B₁ (at which dose enzyme activities were inhibited by about 5%) was essentially comparable to that of a DMSO control (Fig. 1), in that the granules and fibrils were well integrated to form a tight meshwork. It is thus apparent that aflatoxin B₁ at a dose level of 0.1 mg/kg did not induce nucleolar alterations, whereas 0.2 mg/kg was effective in causing nucleolar disorganization.

**DISCUSSION**

Results presented here demonstrate that aflatoxin B₁, administered as a single, sublethal dose (1.0 mg/kg) to rats, causes inhibition of liver DNA-dependent RNA polymerase activity. Characteristics of inhibition were similar when enzyme activation was effected by Mg⁺⁺ or Mn⁺⁺-(NH₄)₂SO₄. Enzyme activity under both conditions of activation was maximally suppressed (65%) for 15 min to 12 hr after the toxin was injected. Inhibition to the extent of
40% for the Mg\(^{++}\)-activated system, and to 10% for the Mn\(^{++}\)\((\text{NH}_4)_2\text{SO}_4\)-activated system, had occurred by 5 min after dosing. The response was reversible in both systems, since enzyme activity returned to control values between 12 to 36 hr.

With respect to time of appearance and maximum degree of inhibition induced by aflatoxin B\(_1\), these results are in general agreement with those obtained under different conditions of RNA polymerase activation by other workers (9, 10). The more marked inhibition (90%) of enzyme activity reported by Clifford and Rees (6) was possibly a result of necrotic changes, since in their experiments an extremely cytotoxic dose (14 mg/kg) of aflatoxin B\(_1\) was used.

Levels of DNA-dependent RNA polymerase activity in liver nuclei from control animals in the present experiments agree well with those reported by Widnell and Tata (27). Maul and Hamilton (14) have shown that the Mg\(^{++}\)-dependent enzyme activity is localized in the nucleolus and that the Mn\(^{++}\)\((\text{NH}_4)_2\text{SO}_4\)-dependent activity is localized in the nucleoplasam, but Jacob et al. (11) have demonstrated the presence of both types of enzyme activities in the nucleolus.

Inhibition of liver nuclear RNA synthesis by aflatoxin B\(_1\) has been reported under several types and conditions. Clifford and Rees (5) showed that an LD\(_{50}\) dose of the toxin given to intact rats inhibited precursor incorporation into liver nuclear RNA to the extent of 80% within 3 hr after dosing. Sporn et al. (20) have found that a comparable dose of the toxin suppressed cytidine incorporation by 92% within 70 min after dosing, and 83% inhibition was still evident 17 hr later.

Recently, Floyd et al. (8) have investigated the short-term effects of aflatoxin B\(_1\) on synthesis of rat liver nucleolar RNA and some of the characteristics of the RNA produced by nucleoli from toxin-treated animals. Rats were dosed with aflatoxin B\(_1\) at levels of 0.05 to 0.5 mg/kg body weight 18 hr after partial (60 to 70%) hepatectomy and killed 30 min later. A dose level of 0.05 mg/kg partially inhibited synthesis of 35, 45, and 55 S RNA, whereas doses of 0.2 mg/kg and larger completely suppressed the synthesis of 45 S RNA. Furthermore, these authors reported that the 0.05 mg/kg dose failed to cause significant alteration in the \(32^P\) base composition of rapidly sedimenting nucleolar RNA after 30 min of treatment.

Taken together, this information clearly indicates that aflatoxin B\(_1\) inhibits ribosomal RNA synthesis. Widnell and Tata (27) have inferred that DNA-dependent RNA polymerase activity assayed in the presence of Mg\(^{++}\) reflects ribosomal RNA synthesis, while the Mn\(^{++}\)\((\text{NH}_4)_2\text{SO}_4\)-activated system indicates synthesis of DNA-like RNA. If this inference is correct, inhibition of the enzyme under both conditions of activation by aflatoxin B\(_1\), together with the observed nucleolar ultrastructural changes, would suggest that synthesis of both ribosomal and DNA-like RNA are inhibited by the toxin.

In regard to cytotoxicity of aflatoxin B\(_1\) as a function of dose, our choice of a dose of 1 mg/kg for a time-course study of RNA polymerase inhibition was based on previous experience with respect to its lack of cytotoxic and lethal potency. Although Floyd et al. (8) used lower absolute doses of aflatoxin B\(_1\) (0.05 to 0.5 mg/kg), their experimental conditions are not exactly comparable to ours because their animals were subjected to partial (67 to 70%) hepatectomy 18 hr prior to use. Thus, the effective toxin dose per unit weight of liver would be considerably greater than the nominal dose per unit body weight. It is therefore difficult to make meaningful comparisons of dose-effect responses in the 2 experiments.

Inhibition of RNA polymerase activity in the present experiments was accompanied by a progressive decline in the nuclear RNA/DNA ratio (Table 1). This ratio was significantly reduced by 15 min after dosing and reached a minimum value (65% of control) 12 hr thereafter. The nuclear DNA content at each time interval was comparable among the NaCl and DMSO control as well as the toxin-treated animals, remaining relatively constant (approximately 0.9 mg DNA/g liver) throughout the 36-hr period. The nuclear RNA content was comparable between the controls (approximately 0.2 mg/g), but that of the toxin-treated animals showed various degrees of decrease from the control values (minimal content equal to 0.1 mg/g). The decline of the RNA/DNA ratio represents a net loss of nuclear RNA. Recovery of enzyme activity to pretreatment levels between 12 and 36 hr was associated with a return of the RNA/DNA ratio to control values.

In general, observed effects of the toxin on RNA polymerase activity as a function of time after dosing were associated with changes in nucleolar ultrastructure. However, the development of nucleolar structural changes did not follow the same time course as enzyme inhibition. Specifically, although enzyme inhibition was maximal within 15 min after dosing, the most extensive segregation of nucleolar components, nucleolar capping, was not detected until 1 hr after treatment. Nonetheless, indications of nucleolar disorganization were observed within 15 min after toxin was injected. These consisted of decrease of prominence of the nucleolonema, with microsegregation of fibrillar and granular components (Fig. 2). Nucleolar capping was observed after 1 hr (Fig. 3). Nucleolar ultrastructural alterations that were clearly evident 12 hr after treatment were not observed after 36 hr; there was reintegration of nucleolar components, and the nucleolus showed no evidence of pathological alterations.

Loss of nuclear RNA is associated temporally with the pattern of alterations in nucleolar ultrastructure inasmuch as these changes indicate nuclear pathological effects induced by aflatoxin B\(_1\). The temporal association of these 2 events is an important consideration. Because the nucleolar components are closely associated with RNA particles (3), alteration of the structural integrity of the nucleolus might lead to increased susceptibility of RNA particles to degradation by ribonuclease which may in part account for nuclear RNA loss. Actinomycin D, which also causes nucleolar capping, has been shown to increase degradation of RNA by ribonuclease (21). Since many biochemical effects of aflatoxin B\(_1\) parallel those of actinomycin D (28), it is possible that nucleolar RNA loss caused by the 2 compounds may result from similar mechanisms.

It has been suggested that 45 S RNA is associated with the
fibrils of the nucleolus and that the pathway of RNA maturation is from the template to the fibrillar and then to the granular component of the nucleolus (3). Actinomycin D inhibits template activity, but it does not prevent movement of the incorporated precursors of RNA from the fibrils to the granules in the nucleolus (3). It does, however, affect the transition of 35 S RNA from a nuclear to a cytoplasmic stage (17). In the present experiments, the loss of prominence of the nucleolonema and microsegregation of nucleolar components 15 mm after dosing suggests the possibility beginning of interference of nucleolar RNA maturation process in addition to maximal RNA polymerase inhibition. Furthermore, these morphological alterations may represent a prelude to nucleolar capping observed at 1 hr after dosing (Fig. 3). RNA polymerase activity is maximally inhibited at this time interval without complete segregation of nucleolar components. Thus, nucleolar capping seemingly is not a prerequisite for maximal enzyme inhibition.

Results of the dose-response experiments reported here demonstrate that inhibition of RNA polymerase, under both conditions of activation, is a linear function of the dose of aflatoxin B1 administered. The correlation coefficients (−0.89 and −0.97) calculated from the dose-activity data indicate a strong correlation between the 2 parameters. These observations also support the hypothesis that the observed effects on enzyme activity are not secondary to nonspecific responses to the toxin, such as cellular necrosis.

Electron microscopic studies in the dose-response experiment (Fig. 4) show that significant nucleolar ultrastructural disorganization occurred when RNA polymerase was inhibited by approximately 20% (by the aflatoxin dose of 0.2 mg/kg). No observable ultrastructural changes occurred at an aflatoxin level of 0.1 mg/kg, a dose that caused approximately 5% inhibition of enzyme activity. These findings indicate that the biochemical parameter may be more sensitive to the toxin, and that biochemical alterations may be detectable earlier than the ultrastructural changes.

Our morphological observations are comparable, in many respects, with those reported by other workers (2, 4, 8, 22, 24). We have, however, extended these findings by establishing a temporal relationship between rat liver RNA polymerase inhibition and nucleolar segregation effected by aflatoxin B1. Although nucleolar capping is associated with maximal RNA polymerase inhibition, this ultimate event of nucleolar segregation is evidently not a temporal prerequisite for maximal RNA polymerase inhibition. In addition, our data indicate that the integration of nucleolar components (as an indicator of recovery from toxic effects) is associated with reversal of RNA polymerase inhibition as demonstrated by the enzyme activity and nucleolar ultrastructure 36 hr after treatment.

Multiple or prolonged administration of aflatoxin B1 to rats in carcinogenic doses does not result in macrosegregation of liver cell nucleoli (23, 24). However, microsegregation of nucleolar components has been shown to occur between 2 weeks after the commencement and 4 weeks after withdrawal of carcinogenic treatment (23). Although suppression of RNA polymerase activity has been demonstrated during N-nitrosomorpholine carcinogenesis (12) and our experiments have shown the temporal relationship between nucleolar microsegregation and RNA polymerase inhibition, it is not possible at present to assign the roles of these cellular responses in aflatoxin B1 carcinogenesis.

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REFERENCES


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Figs. 1 to 4 are electron micrographs of rat liver nucleoli. Tissues were fixed in glutaraldehyde and postfixed in OsO4. Silver sections were stained in uranyl acetate and lead citrate.

Fig. 1. Rat liver nucleolus after a single i.p. injection of 0.05 ml dimethyl sulfoxide. The fibrils (f) form a coarse strand that branches and anastomoses to form a tight meshwork with the granules (g). Nucleolus-associated chromatin (Ch) and perichromatinic granules (p) are present. X 63,610.

Fig. 2. Rat liver nucleolus 15 min after 1 mg/kg of aflatoxin B1. Decrease in prominence of nucleolonema and microsegregation of nucleolar components are observed. X 63, 610. /, fibrils; g, granules.

Fig. 3. Rat liver nucleolus 1 hr after 1 mg/kg aflatoxin B1. Nucleolar capping is demonstrated. The granular (g) and fibrillar (f) components are separated into distinct areas. Ch, nucleolus-associated chromatin. X 113, 540.

Fig. 4. Rat liver nucleolus 15 min after 0.2 mg/kg aflatoxin B1. Nucleolar components are not well integrated. These are more evident in the lower nucleolus. X 36, 770.
Aflatoxin Effects on Nuclei
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