[CANCER RESEARCH 42, 1876–1880, May 1982]
0008-5472/82/0042-0000$02.00

Inhibition of Mitochondrial Protein Synthesis during Early Stages of Aflatoxin B_1-induced Hepatocarcinogenesis[1]

Narayan K. Bhat, John K. Emeh,[2] Banavadi G. Niranjan, and Narayan G. Avadhani[3]

Laboratories of Biochemistry, Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104

ABSTRACT

Experiments were designed to determine the *in vivo* effects of a single 6-mg/kg dose aflatoxin B_1 on rat liver mitochondrial transcription and translation processes. With the use of intact hepatocytes and also a highly active mitoplast system for incorporation, it was observed that both mitochondrial transcription and translation activities are inhibited progressively even after 24 hr of carcinogen administration. Electrophoretic patterns of mitochondrial translation products show some qualitative changes during early periods of carcinogen administration. At later stages (>12 hr), however, there is a general inhibition of many of the products, although by this time there is a qualitative and quantitative recovery in the synthesis of mitochondrial proteins imported from the cytoplasm. These results, along with the data showing considerably high levels of aflatoxin B_1 binding to mitochondrial DNA suggest that mitochondrial genetic system is one of the direct targets during experimental carcinogenesis.

INTRODUCTION

The role of mitochondria in the production and maintenance of cancer phenotype has been the subject of argument ever since the pioneering work of Warburg (27), which showed fundamental changes in the metabolic patterns of tumor tissues. Over the years, a number of studies have demonstrated altered mitochondrial content (8, 13), structure, and function (11, 12, 28) in a variety of tumor cells. It has been shown that mitochondria from tumor cells have altered ultrastructural organization, membrane composition, abnormal ion transport, and altered biochemical properties (for a discussion, see Ref. 22). Leukemic leukocytes and several solid tumors have been shown to contain unusual types of oligomeric and catenated forms of mitochondrial DNA (9, 18, 29). Another line of investigation suggests that mitochondria may be one of the cellular targets of attack by carcinogenic agents (22). Substantial amounts of carcinogens administered to experimental animals (2, 31) or incubated with tissue culture cells (4) not only are localized in the mitochondrial fraction but also are covalently bound to mitochondrial nucleic acids. Wunderlich *et al.* (31) found that hepatic carcinogen such as *N*-nitrosodimethylamine preferentially methylates mitochondrial DNA under both *in vivo* and *in vitro* conditions. Furthermore, experiments of Baker and Weinstein (4) showed that the dihydrodiol-epoxide derivative of benzo(*a*)pyrene covalently modifies mitochondrial DNA at efficiencies 40 to 90 times greater than those for the nuclear DNA. Recent experiments in our laboratory showed the presence of a unique monooxygenase system, different from the microsomal activity in rat liver mitochondria, which can activate hepatic carcinogen AFB_1[4] into electrophilic reactive forms (19). The activated components bind to mitochondrial DNA, RNA, and proteins (19, 20). All of these results taken together suggest that the mitochondrial genetic system may be the direct and possibly important target of carcinogens. In an attempt to verify this possibility, we have now studied the extent of AFB_1 attack on the mitochondrial genome and the resultant effects on mitochondrial transcription and translation processes under *in vivo* conditions.

MATERIALS AND METHODS

Materials. Amino acids, nucleotide triphosphates, chloramphenicol, cycloheximide, D-mannitol, and bovine serum albumin were purchased from Sigma Chemical Co., St. Louis, Mo. RNase-free sucrose and electrophoresis grade SDS were from Polysciences Inc., Warrington, Pa. Electrophoresis grade acrylamide, bisacrylamide and other reagents used for electrophoresis were from Bio-Rad, Richmond, Calif. AFB_1 was from Calbiochem/Behring Corp., La Jolla, Calif. Tissue culture media and reagents were from Grand Island Biological Co., Grand Island, N.Y. [^{35}S]Methionine (1200 Ci/mmol) was from Amersham Radiochemicals Co. [^{3}H]GTP (15 Ci/mmol) and [^{3}H]CTP (20 Ci/mmol) were from New England Nuclear, Boston, Mass. All other chemicals used were of analytical grade.

Animals and Carcinogen Administration. In all the experiments, 5- to 7-week-old male Sprague-Dawley rats (150 to 200 g) were used. AFB_1 dissolved in dimethyl sulfoxide was injected i.p. at a dose of 6 mg/kg. The control animals received equal volumes of dimethyl sulfoxide. The treated and untreated animals were anesthetized with ether and sacrificed by cervical fracture. The livers were either removed and used for isolating mitochondria or perfused *in situ* for preparing hepatocytes.

Preparation of Mitochondria. Livers were washed free of blood clots with ice-cold 0.15 M NaCl and minced with a scissor into 2- to 3-mm slices. The tissue slices were homogenized in mitochondrial isolation buffer (2 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.5-0.22 M D-mannitol-0.07 M sucrose-1 mM EDTA-bovine serum albumin, 0.5 mg/ml) with 3 strokes of a loosely fitted (0.02-cm clearance) Teflon homogenizer at 1000 to 1200 rpm. The homogenate was made to 10% (w/v) with mitochondrial isolation buffer. Mitochondria were separated by the differential centrifugation method described before (3). The mitoplasts were prepared by the digitonin method as described before (25), washed once with mitochondrial isolation buffer, and used for RNA or protein synthesis.

Protein Synthesis with Mitoplasts. The details of the method used for protein synthesis with isolated mitoplasts have been described elsewhere (6, 7). Briefly, mitoplasts were suspended in protein synthesis buffer (15 mM Tris-HCl), pH 7.4; 65 mM KCl; 6 mM $Mg(CH_3COO)_2$; 5 mM 2-mercaptoethanol; 4 mM potassium phosphate buffer, pH 7.4; and 0.14 M sucrose at a concentration of 10 mg protein per ml and

[1] Supported by NIH Grant CA-22762.

[2] Recipient of a fellowship from the Nigerian Institute for Trypanosomiasis Research. Present address: Nigerian Institute for Trypanosomiasis Research, Vom, Jos, Nigeria.

[3] To whom requests for reprints should be addressed.

Received October 12, 1980; accepted February 8, 1982.

[4] The abbreviations used are: AFB_1, aflatoxin B_1; SDS, sodium dodecyl sulfate.

supplemented with 2 mM ATP; 2 mM GTP; 5 mM creatine phosphate; 4 mM pyruvate; creatine phosphokinase, 0.2 mg/ml; and 100 μM concentrations each of 19 L-amino acids except methionine and cycloheximide, 300 μg/ml. The mixture was incubated for 5 min at 35° with or without added chloramphenicol. Protein synthesis was initiated with [^{35}S]methionine (500 μCi/ml; 1000 Ci/mol), and the labeling was continued for the required length of time. Aliquots of 10 μl were removed at intervals and used for determining the hot CCl_3COOH-insoluble counts (6). At the end of incubation, remaining mitoplasts were pelleted at 10,000 × *g* for 10 min, washed once with mitochondrial isolation buffer and kept frozen at −70° in the presence of 3 μg each of leupeptin and pepstatin as protease inhibitors.

RNA Synthesis with Mitoplasts. The method for RNA synthesis was modified from Avadhani *et al.* (1). Mitoplasts were suspended in protein synthesis buffer at a concentration of 3 mg protein per ml and supplemented with 2 mM ATP, 2 mM UTP, 0.5 nM GTP, 0.5 nM CTP, 5 mM malate, 5 mM creatine, creatine phosphokinase (400 μg/ml), spermidine (20 μg/ml), and 100 μM concentrations each of 20 amino acids. The mixture was preincubated at 37° for 3 min, and RNA synthesis was initiated with 20-μCi/ml concentrations each of [^{3}H]GTP and [^{3}H]CTP. The incorporation was continued for 40 min. At intervals, 10-μl samples were withdrawn and used for determining cold trichloroacetic acid-insoluble counts (1).

Protein Synthesis with Hepatocytes. Hepatocytes were prepared by perfusion with collagenase buffer, as described by Williamson *et al.* (30), except that Hanks' medium was used for perfusion as well as for releasing the cells (10). The details of protein synthesis using [^{35}S]methionine were as described elsewhere (20). Unless otherwise mentioned, protein synthesis was carried out for 120 min in either the presence or the absence of added inhibitors such as chloramphenicol and cycloheximide. Aliquots (10 μl) were taken out at intervals and used for determining the hot CCl_3COOH-insoluble cpm as in the case of mitoplasts (6).

Electrophoretic Analysis of Proteins. Mitochondrial samples were dissolved in a lysis buffer containing 4% SDS and 10% 2-mercaptoethanol and dissociated by heating at 90° for 2 min (6). Electrophoresis was carried out on 8 to 16% gradient polyacrylamide slab gels containing 0.2% SDS, using the buffer system of Laemmli (14). The gels were stained with Coomassie blue, destained in 30% methanol-10% acetic acid, and processed for fluorography with En^{3}hance (New England Nuclear).

Other Procedures. Protein was estimated by the method of Lowry *et al.* (15). For radioactivity determinations, the samples were counted with a 10-ml Cab-o-Sil scintillation mixture in an Intertechnique SL-4000 counter.

RESULTS

To determine the direct and indirect effects of AFB$_1$ on mitochondrial biosynthetic and genetic processes, we have carried out experiments with intact hepatocytes and with isolated mitochondrial particles. As shown in Chart 1, the hepatocyte system used in these studies is highly active in protein synthesis. Addition of cycloheximide (300 μg/ml) inhibits the [^{35}S]methionine incorporation by about 87%. Addition of cycloheximide and chloramphenicol together results in >96% inhibition. In separate experiments reported elsewhere (7), we have shown that the cycloheximide-resistant and chloramphenicol-sensitive incorporation, corresponding to about 8 to 12% of total cell synthesis in rat hepatocytes, represents true mitochondrial-specific protein synthesis.

The system for RNA and protein synthesis with isolated mitochondria involves the incubation of digitonin-washed mitoplasts in a hypoosmotic medium, which permits efficient incorporation (6). The kinetics of [^{35}S]methionine incorporation by mitoplasts isolated from control liver has been presented in Chart 2*A*. Addition of chloramphenicol, a specific inhibitor of mitochondrial protein synthesis, results in a near-total inhibition of incorporation. Similarly, the system incorporates [^{3}H]GTP and [^{3}H]CTP linearly up to about 40 to 60 min (Chart 2*B*), and the incorporation is sensitive to low amounts (0.5 μg/ml) of ethidium bromide as reported for other mitochondrial systems (17, 23).

The patterns of mitochondrial-specific translation and transcription after various time intervals of AFB$_1$ (6 mg/kg) are presented in Chart 3. Experiments with isolated mitoplasts show a progressive inhibition of mitochondrial translation up to 60 hr after AFB$_1$ administration. The extent of translational inhibition observed with the mitoplast system is nearly identical to the cycloheximide-resistant incorporation, which is characteristic of mitochondrial-specific translation in intact hepatocytes (Chart 3). Similarly, the mitochondrial transcription activity as estimated by [^{3}H]GTP and [^{3}H]CTP incorporation by isolated mitoplasts resembles the pattern of translation inhibition following AFB$_1$ administration. A previous report from this laboratory showed that nuclear transcription and cytoplasmic

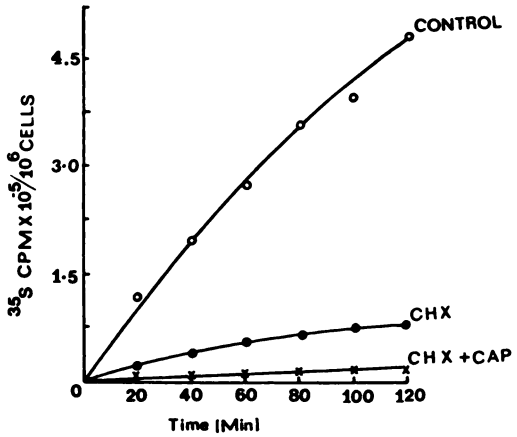

Chart 1. Kinetics of [^{35}S]methionine incorporation by isolated hepatocytes. Hepatocytes prepared from control rats were used for incorporation using [^{35}S]methionine (100 μCi/ml) as described in "Materials and Methods." ○, control without added inhibitor; ●, cycloheximide (*CHX*) (300 μg/ml); ×, cycloheximide (300 μg/ml) + chloramphenicol (*CAP*) (500 μg/ml).

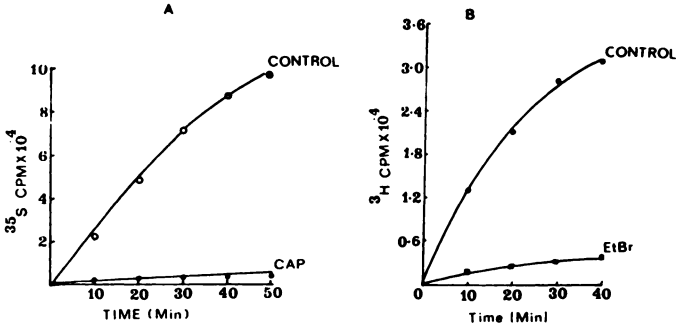

Chart 2. Protein and RNA synthesis with isolated mitoplasts. Mitoplasts were prepared from control rat liver as described in "Materials and Methods." In *A*, mitoplasts were suspended in protein synthesis buffer at a final concentration of 10 mg protein per ml and incubated with [^{35}S]methionine (150 μCi/ml). Ten-μl samples were withdrawn at each time point for determining hot CCl_3COOH-insoluble cpm. Chloramphenicol (*CAP*) when added was at 500 μg/ml. *B*, RNA synthesis was carried out with 20-μCi/ml doses each of [^{3}H]GTP and [^{3}H]CTP. The final concentration of mitoplasts in the reaction mixture was 3 mg/ml. *Points* represent cold CCl_3COOH-insoluble cpm in 10-μl aliquots. Ethidium bromide (*EtBr*) when added was at 0.5 μg/ml.

translation activities in intact hepatocytes following a drug dose of 6 mg/kg are progressively inhibited up to about 6 to 9 hr (10). It was also observed that both of these activities are recovered to control level by about 12 hr and reach a near 150 to 220% level by 24 hr (10). Thus, in contrast to the recovery process seen in the nuclear and cytoplasmic activities between 9 and 24 hr, the mitochondrial biosynthesis processes remain inhibited at least up to 60 hr after carcinogen administration.

To determine the nature of AFB_1-induced inhibition, the ^{35}S-labeled mitochondrial translation products were analyzed by electrophoresis on 8 to 16% polyacrylamide gels under dissociating conditions. The autoradiogram presented in Fig. 1 shows that rat liver mitochondria synthesize about 20 polypeptides in the size range of about 8×10^3 to 13×10^4 daltons. The pattern of mitochondrial translation, 6 hr after AFB_1 administration, shows several qualitative differences. Some unusual products in the size range of 10, 4.5, and 1.8×10^4 daltons are noticeable. The 12-hr pattern shows marked inhibition of several products, and the inhibition appears to be general. Although not shown here, the electrophoretic patterns of proteins after 24 and 58 hr of AFB_1 treatment show pronounced inhibition.

To determine if the AFB_1-induced changes in mitochondrial translation pattern are due to extramitochondrial effects or direct effects on mitochondrial genetic and biosynthetic systems, we have carried out 2 types of experiments.

In the first set of experiments, the effects of AFB_1 on the labeling of mitochondrial polypeptides contributed by the cytoplasmic translation system were studied. As shown in Fig. 2, hepatocytes from rats treated with AFB_1 (6 mg/kg) were labeled with [^{35}S]methionine in the presence of chloramphenicol, and the mitochondrial particles isolated from these cells were electrophoresed on 8 to 16% gradient gels under highly dissociating conditions. In agreement with our previous report on the pattern of cytoplasmic translation inhibition by AFB_1 (10), there is a marked inhibition of cytoplasmically imported mitochondrial proteins after 3 hr of drug treatment (Fig. 2*A*). This inhibitory effect appears to be recovered by 12 hr (Fig. 2*B*), and the 24-hr pattern (Fig. 2*C*) compares with the control pattern (Fig. 2*D*) both qualitatively and quantitatively.

In the second set of experiments, we have determined the levels of bound AFB_1 to mitochondrial and nuclear DNA at

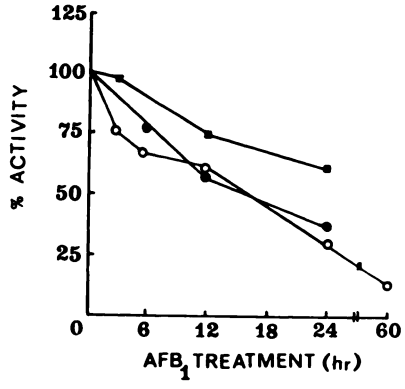

Chart 3. The time course of inhibition of mitochondrial transcription and translation by a single dose of AFB_1. Livers from AFB_1-treated animals were used for preparing mitoplasts or hepatocytes as described in "Materials and Methods." ●, mitochondrial protein synthesis with intact hepatocytes. Hepatocytes were labeled with [^{35}S]methionine for 120 min in the presence of cycloheximide (300 μg/ml) to suppress cytoplasmic translation. Other details were as described in Chart 1. ○, mitochondrial translation with mitoplasts. Mitoplasts were labeled for 60 min with [^{35}S]methionine as described in *A*. The values obtained with control mitoplasts were regarded as 100% activity. ■, mitochondrial RNA synthesis with isolated mitoplasts. Mitoplasts were labeled for 40 min with 20-μCi/ml amounts each of [^3H]GTP and [^3H]CTP as described in Chart 2*B*. Values obtained with control mitoplasts (*Chart 2B*) were considered as 100% activity.

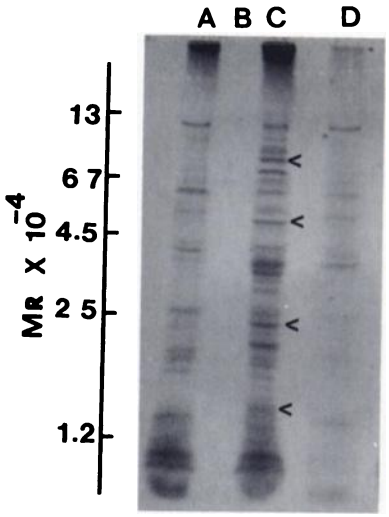

Fig. 1. Effects of AFB_1 on mitochondrial translation products. Isolated mitoplasts were labeled with [^{35}S]methionine for 60 min as described in "Materials and Methods." Samples containing about 10^5 cpm were dissociated by heating at 90° for 2 min in the presence of 10% 2-mercaptoethanol and 4% SDS and electrophoresed on 8 to 16% gradient polyacrylamide gels. In order to account for the AFB_1-induced inhibition of incorporation, the amount of protein used in AFB_1-treated samples (160 μg) was roughly twice as much as the control (80 μg). *A*, control mitoplasts; *B*, control mitoplasts labeled in the presence of chloramphenicol (500 μg/ml); *C*, mitoplasts from rats treated with a single dose of AFB_1 (6 mg/kg) for 6 hr; *D*, mitoplasts from rats treated with a single dose of AFB_1 (6 mg/kg) for 12 hr. *Arrows* in *C*, qualitative changes in mitochondrial translation products. Details of fluorography and autoradiography were as described in "Materials and Methods."

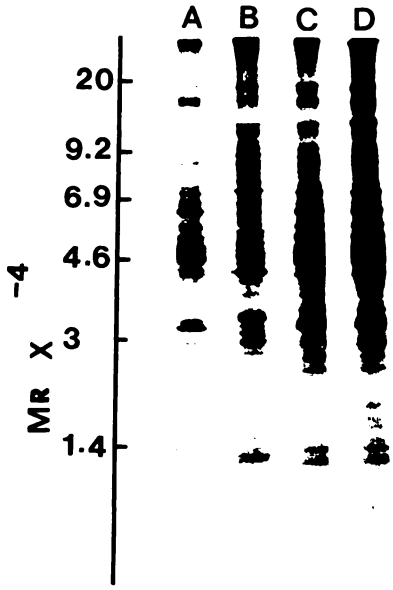

Fig. 2. Effects of AFB_1 on mitochondrial protein imported from the cytoplasm. Hepatocytes were prepared from control and AFB_1-treated animals and labeled with [^{35}S]methionine for 120 min in the presence of chloramphenicol (500 μg/ml). Mitoplasts were prepared and electrophoresed on 8 to 16% gradient gels after dissociation in the presence of 10% 2-mercaptoethanol and 4% SDS as described in Fig. 1. *A*, 3-hr AFB_1; *B*, 12-hr AFB_1; *C*, 24-hr AFB_1; *D*, control. In each case, 50 μg protein were used for electrophoresis.

various times after the carcinogen administration. As shown in Table 1, nuclear DNA contains about 3 AFB_1 adducts/10^7 daltons after 3 hr, whereas the mitochondrial genome at this time period contains about 9 adducts/10^7 daltons DNA. The number of AFB_1 adducts in the nuclear DNA declines steadily to reach about 0.3/10^7 daltons DNA at 24 hr. In contrast, the level of bound AFB_1 in the mitochondrial DNA remains nearly the same even after 24 hr. Results of these experiments together suggest that prolonged inhibition of mitochondrial translation and altered mitochondrial polypeptide pattern may result from the direct effects of AFB_1 on the mitochondrial genetic system, although some indirect effects involving the nuclear gene products cannot be overruled.

DISCUSSION

Recent studies in our laboratory showed that nuclear transcription and cytoplasmic translation activities following a single dose of AFB_1 exhibit typical inhibitory and recovery patterns (10). After nearly 80% inhibition of both of the processes at 6 to 9 hr, the activities reach a peak of about 220% level at 24 hr after AFB_1 administration (10). In view of this, the patterns of mitochondrial transcription and translation activities following AFB_1 administration are markedly different (Chart 3), in that both of these activities remain inhibited without any apparent recovery up to about 24 to 60 hr.

The analysis of mitochondrial translation products on gradient SDS-polyacrylamide gels under highly dissociating conditions shows that control mitochondria synthesize about 20 to 22 polypeptides including some in the range of 13×10^4 daltons. Recently, we have shown that all of these polypeptide species represent true mitochondrial translation products, although some may be the precursors of smaller polypeptides (6). The polypeptides synthesized after 6 hr of AFB_1 treatment contain several unusual species not seen in the control samples (Fig. 1*C*). These polypeptides may be originated from altered processing of mitochondrial transcripts or are probably due to defective processing of the precursor polypeptides. At longer duration of AFB_1 treatment, however, there is increasing inhibition of several products. The inhibition appears to be general and may result from the inhibition of mitochondrial transcription process.

It is now reasonably well established that most of the mitochondrial proteins are imported from the cytoplasm (24). These imported proteins are believed to be coded by the nuclear genes (24, 26). The mitochondrial genetic system, on the other hand, contributes to only <10% of the proteins, which are highly essential for the assembly of mitochondrial membranes and their function (24, 26). Although not conclusively proven, it is widely believed that most, if not all, of mitochondrially translated proteins are coded for by the mitochondrial genome (26). The observed inhibition of mitochondrial translation products may therefore be due to the direct attack on mitochondrial genetic system. Alternatively, the inhibition may be due to decreased cytoplasmic synthesis of mitochondrial biosynthetic enzymes, which are known to be imported from the cytoplasm (24). Continued inhibition of mitochondrial activity even after full recovery of cytoplasmic translation at 24 hr (Chart 3; Ref. 10, 21) suggests that this is a less likely possibility. This view is further supported by experiments showing a qualitative and quantitative recovery in the labeling patterns of cytoplasmically imported mitochondrial proteins at 24 hr after carcinogen administration (Fig. 2, *C* and *D*). Support for the direct involvement of mitochondrial genetic system comes from experiments on the levels of binding of AFB_1 to nuclear and mitochondrial DNA (Table 1). As shown for a number of carcinogens (16), the level of bound AFB_1 in the nuclear DNA reduces gradually to reach 0.3 adduct/10^7 daltons DNA at 24 hr. Thus, the recovery in the nuclear transcription and cytoplasmic translation may reflect upon the removal of AFB_1 adducts and the repair of DNA (15). Contrary to this, the level of bound AFB_1 in the mitochondrial genome remains nearly constant even up to 24 hr, which is possibly due to the lack of repair in this organelle system (4). It should also be noted that the number of adducts per 10^7 daltons mitochondrial DNA is about 3 to 4 times higher than the level observed for nuclear DNA. These results are consistent with previous reports showing relatively higher levels of binding of various carcinogens to mitochondrial DNA (4, 31). Thus, although it is possible that effects of AFB_1 on the nuclear genes may somehow contribute to altered mitochondrial activity, persistent inhibition of mitochondrial transcription and translation activities beyond 12 to 24 hr of carcinogen administration appears to be largely due to the direct attack on the organelle genetic system.

In summary, we have presented data which demonstrate that *in vivo* administration of AFB_1 leads to a prolonged inhibition of mitochondrial biosynthetic activities. These observations confirm and extend the view that mitochondrial DNA is a direct target for attack by chemical carcinogens (4, 19, 22, 31). It has already been shown that AFB_1 causes a long-term inhibition of various mitochondrial functions including the oxidative phosphorylation (5, 20). Although the precise role of mitochondrial genes in the carcinogenic process remains unknown, it is possible that altered mitochondrial biosynthetic processes affecting mitochondrial oxidative function and varied metabolic activities of the cell may directly or indirectly contribute to the neoplasmic outcome.

Table 1

Relative levels of binding of AFB_1 to nuclear and mitochondrial DNA

Rats were given i.p. injections of 6 mg of [^{3}H]AFB_1 per kg (30 μCi/2.9 μmol). After indicated time periods, livers were removed and used for isolating the mitoplasts (19) and nuclei (10) as described before. Mitochondrial-specific circular DNA was isolated by density banding in CsCl-ethidium bromide gradients (9, 19). Nuclear DNA was isolated by the phenol-cresol method and was further purified by banding in CsCl-ethidium bromide gradients (9). The DNA samples were freed of ethidium bromide and CsCl (19), and bound radioactivity in the DNA was estimated by counting aliquots with 10-ml Cab-o-Sil scintillation mixture as before (19). The values were derived from 3 estimates.

Single dose AFB_1 treatment (hr)	No. of adducts/10^7 daltons DNA	
	Nuclear DNA	Mitochondrial DNA
3	3.2 ± 0.4[a]	10.2 ± 1.3
6	2.4 ± 0.6	11.1 ± 1.5
12	1.9 ± 0.5	9.8 ± 0.6
24	0.3 ± 0.2	10.1 ± 1.4

[a] Mean ± S.E.

ACKNOWLEDGMENTS

The authors are thankful to Nina Leinwand for helping with the preparation of the manuscript and to Joann Shepard and Aruna Bhat for technical assistance.

REFERENCES

1. Avadhani, N. G., Batula, N., and Rutman, R. J. Messenger ribonucleic acid

metabolism in mammalian mitochondria. Origin of ethidium bromide resistant polyadenylic acid containing ribonucleic acid in Ehrlich ascites mitochondria. Biochemistry, *12:* 4122–4129, 1973.
2. Avadhani, N. G., Hansel, G., Ritter, C., and Rutman, R. J. Comparison of the effects of nitrogen mustard on the functional properties of mitochondria from sensitive and resistant strains of Ehrlich ascites tumors. Cancer Biochem. Biophys., *1:* 167–174, 1976.
3. Avadhani, N. G., Lewis, F. S., and Rutman, R. J. Messenger ribonucleic acid metabolism in mammalian mitochondria. Quantitative aspects of structural information coded by the mitochondrial genome. Biochemistry, *13:* 4638–4645, 1974.
4. Backer, J. M., and Weinstein, I. B. Mitochondrial DNA is a major cellular target for dihydrodiol-epoxide derivative of benzo(*a*)pyrene. Science (Wash. D. C.), *209:* 297–299, 1980.
5. Bai, N. J., Pai, M. R., and Venkitasubramanian, T. A. Mitochondrial function in aflatoxin toxicity. Indian J. Biochem. Biophys., *14:* 347–349, 1977.
6. Bhat, N. K., Niranjan, B. G., and Avadhani, N. G. Complexity of mitochondrial translation products in mammalian cell. Biochem. Biophys. Res. Commun., *103:* 621–628, 1981.
7. Bhat, N. K., Niranjan, B. G., and Avadhani, N. G. Variant translation products in mammalian mitochondria. J. Cell Biol., *91:* 286, 1981.
8. Cederbaum, A. I., Becker, F. F., and Rubin, E. Ethanol metabolism by transplantable hepatocellular carcinoma. Role of microsomes and mitochondria. *J. Biol. Chem., 251:* 5366–5374, 1976.
9. Clayton, D. A., and Vinograd, J. Complex mitochondrial DNA in leukemic and normal myeloid cell. Proc. Natl. Acad. Sci. U. S. A., *62:* 77–1084, 1969.
10. Emeh, J. K., Niranjan, B. G., Bhat, N. K., and Avadhani, N. G. Modulation of hepatic transcription and translation during early stages of aflatoxin B_1 carcinogenesis. Carcinogenesis, *2:* 373–378, 1981.
11. Feo, F., Bonelli, G., Canuto, R. A., and Garcia, R. Further observations on the effects of trypsin on the volume and functions of mitochondria isolated from normal liver and AH-130 Yoshida ascites hepatoma. Cancer Res., *33:* 1804–1812, 1973.
12. Hackenbrock, C. R., Rehen, R. G., Weinbach, E. C., and Lemaster, J. J. Oxidative phosphorylation and ultrastructural transformations in mitochondria in the intact ascites tumor cell. J. Cell Biol., *51:* 123–137, 1971.
13. Howatson, A. F., and Ham, A. W. Electron microscopy study of sections of two rat liver tumors. Cancer Res., *15:* 62–69, 1955.
14. Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature (Lond.), *227:* 680–685, 1970.
15. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. Protein measurement with the Folin phenol reagent. J. Biol. Chem., *193:* 265–275, 1951.
16. Mahler, V. M., and McCormick, J. J. DNA repair and carcinogenesis. *In*: P. L. Grover (ed.), Chemical Carcinogenesis and DNA, Vol. 2, pp. 133–158. Boca Raton, Fla.: CRC Press, Inc., 1978.
17. Maitra, U., Nakata, Y., and Hurwitz, J. The role of deoxyribonucleic acid in ribonucleic acid synthesis. A study of the initiation of ribonucleic acid synthesis. J. Biol. Chem., *242:* 4908–4918, 1967.
18. Nass, M. M. K. Mitochondrial DNA. Advances, problems, and goals. Science (Wash. D. C.), *165:* 25–35, 1969.
19. Niranjan, B. G., and Avadhani, N. G. Activation of aflatoxin B_1 by a monooxygenase system localized in rat liver mitochondria. J. Biol. Chem., *255:* 6575–6578, 1980.
20. Niranjan, B. G., and Avadhani, N. G. Tissue specificity of mitochondrial monooxygenase system for aflatoxin B_1 activation. Biochem. Biophys. Res. Commun., *94:* 1021–1026, 1980.
21. Obidoa, O., and Siddiqui, T. H. Aflatoxin inhibition of avian hepatic mitochondria. Biochem. Pharmacol., *27:* 547–550, 1977.
22. Pedersen, P. L. Tumor mitochondria and the bioenergetics of cancer cells. Prog. Exp. Tumor Res., *22:* 190–274, 1980.
23. Penman, S., Fan, H., Perlman, S., Rosbash, M., Weinberg, R., and Zylber, E. Distinct RNA synthesis systems of the HeLa cell. Cold Spring Harbor Symp. Quant. Biol., *35:* 561–575, 1970.
24. Schatz, G., and Mason, T. L. The biosynthesis of mitochondrial proteins. Annu. Rev. Biochem., *43:* 51–87, 1974.
25. Schnaitman, C., and Greenawalt, J. W. Enzymatic properties of the inner and outer membranes of rat liver mitochondria. J. Cell Biol., *38:* 158–175, 1968.
26. Tzagoloff, A., Macino, G., and Sebald, W. Mitochondrial genes and translation products. Annu. Rev. Biochem., *48:* 419–441, 1979.
27. Warburg, O. Metabolism of Tumors, London: Arnold Constable, 1930.
28. White, M. T., Arya, D. V., and Tewari, K. K. Biochemical properties of neoplastic cell mitochondria. J. Natl. Cancer Inst., *53:* 553–558, 1974.
29. White, M. T., Wagner, E. K., and Tewari, K. K. Physico chemical characterization of Novikoff hepatoma mitochondrial DNA. Cancer Res., *35:* 873–879, 1975.
30. Williamson, J. R., Browning, E. T., and Scholz, R. Control mechanisms of gluconeogenesis in perfused rat liver. J. Biol. Chem., *244:* 4607–4616, 1969.
31. Wunderlich, V., Tetzlatt, F., and Gratti, A. Studies on nitrosodimethylamine preferential methylation of mitochondrial DNA in rats and hamsters. Chem.-Biol. Interact., *4:* 81–89, 1972.