HEPATIC DNA REPAIR SYNTHESIS IN RATS FED 3'-METHYL-4-DIMETHYLAMINOAZOBENZENE

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SUMMARY

Rats, whose liver DNA was prelabeled with BdUrd-^{14}C, were placed on a diet containing 0.05% of the hepatic carcinogen 3'-methyl-4-dimethylaminoazobenzene. At various times during the second day on the carcinogenic diet animals received an injection of dThd-^{3}H and were sacrificed 1 hr later, hepatic DNA was extracted and analyzed by density equilibrium centrifugation in alkaline CsCl gradients. The results of these studies indicate that non-semiconservative DNA synthesis (DNA repair synthesis is an early consequence of carcinogen exposure and that the amount of DNA repair synthesis occurring at a given time depends, in part, on the animals feeding schedule.

INTRODUCTION

DNA repair synthesis (excision repair) has been extensively examined in mammalian cells in culture following exposure to ultraviolet-irradiation (1, 2), X-irradiation (3), and chemical carcinogens (4). A positive correlation has been made between the oncogenicity of derivatives of 4-nitroquinoline-1-oxide and their capacity of provoke DNA repair synthesis (5). The interest in the possible relationship between carcinogenesis and DNA repair has been stimulated by reports that cells from humans with the hereditary high-cancer disease xeroderma pigmentosum have a capacity for DNA repair that is less than that of normal human cells (6). However, only a few reports have dealt with the question of DNA repair in the intact animal. The first evidence that a chemical carcinogen (3'-MeDAB[1]) could evoke DNA repair synthesis in a target organ *in vivo* was reported in 1972 (7). This study was subsequently confirmed in a series of reports (8, 9) indicating that exposure of rats

[1]Abbreviations: 3'-MeDAB, 3'-methyl-4-dimethylaminoazobenzene; dThd-^{3}H, thymidine-methyl-^{3}H; BdUrd-^{14}C, 5-bromodeoxyuridine-2-^{14}C.

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in vivo to a number of alkylating agents results in hepatic DNA repair synthesis.

There were some methodology problems associated with the use of alkaline sucrose gradient analysis of DNA in these in vivo studies; in the first experiments (7) the extraction procedure results in shearing of the DNA which decreases the sensitivity of the method, and in the second series of experiments (8, 9) control DNA rapidly sedimented to the bottom of the gradients indicating that some aggregation may have occurred. Therefore, it was deemed important to attempt an alternative approach to the study of DNA repair synthesis in vivo. This report deals with an assessment of DNA repair synthesis in the livers of animals fed the hepatic carcinogen 3'-MeDAB by monitoring the extent of non-semiconservative DNA synthesis.

MATERIALS AND METHODS

Animals and Carcinogenic Diet: The procedures employed were similar to those previously described (7). Male Sprague-Dawley rats, weighing approximately 175 g obtained from Spartan Research Animals, Inc., Haslett, Mich. were used for these studies. A control basal diet (10) (carcinogenic basal diet) was purchased from General Biochemicals, Chagrin Falls, Ohio. This control diet was supplemented with 0.05% (w/w) 3'-MeDAB. Although the rats were allowed water ad libitum, access to both basal and 3'-MeDAB-containing diets was restricted to one 8 hr period (8:30 A.M. to 4:30 P.M.) each day by use of the controlled feeding and lighting schedule described as "8+16" (11, 12). Animals were maintained on the control basal diet for 5 to 7 days prior to being switched to the 3'-MeDAB diet.

Radioactive Precursors: BdUrd-^{14}C and dThd-^{3}H with a specific radioactivity of 50 mCi/mMole and 6.7 Ci/mMole, respectively, were purchased from New England Nuclear Corp., Boston, Mass.

Prelabeling Hepatic DNA with BdUrd-^{14}C During Liver Regeneration: The procedure used to prelabel hepatic DNA with BdUrd-^{14}C during liver regeneration following a 70% partial hepatectomy was similar to that previously employed to prelabel hepatic DNA

with dThd-^{3}H (7). The animals received an i.p. injection of BdUrd-^{14}C (1.5 μCi, 4 μmoles/100 gm) at 22, 23, 24, 45, 46, and 47 hr after the operation. These times for injection were chosen to correspond to the 2 peak periods of dThd-^{3}H incorporation following partial hepatectomy (13). The animals were approximately 4 weeks old at the time of the partial hepatectomy, and were allowed 3 weeks to recover prior to being used for experiments.

Extraction of DNA from Hepatic Nuclei: DNA was extracted from a total liver nuclei fraction. The procedures for preparing the nuclei fraction, extraction of DNA and determination of DNA conc were the same as those previously described for the preparation of DNA for sucrose gradient sedimentation studies (7). DNA was dissolved in a solution of 0.15 M NaCl - 0.015 M sodium citrate (SSC). Prior to alkaline CsCl equilibrium centrifugation DNA solutions were sonicated to reduce the molecular weight to 2 x 10^{5} or less (2).

Alkaline CsCl Density Equilibrium Centrifugation of BdUrd-2-^{14}C-Containing Rat Liver DNA: Solid CsCl (purchased from Schwarz Mann, Orangeburg, New York) was added to 3.0 ml of SSC, containing 150 to 250 μg of DNA, the pH of the solution was adjusted to 12.5 with 2 N NaOH and the density was adjusted to 1.70 to 1.71. The density was determined by measuring the refractive index using an Abbe-3L Refractimeter (Bausch and Lomb, Inc., Rochester, N.Y.). The preparations were placed in polyallomer tubes and density equilibration was obtained by centrifuging at 35,000 RPM (87,000 x g) in a type 40.3 Spinco rotor (Beckman Instrument Co., Palo Alto, Calif.) for 48 hr at 25°. Two drop fractions were collected from the bottom of each tube.

Determination of Radioactivity Incorporated Into DNA: One ml of 2.5 N HCl was added to each fraction collected from the gradients and they were then placed in a boiling water bath for 20 min prior to the addition of scintillation fluor (Multisol, purchased from Isolab Inc., Akron, Ohio).

RESULTS

All of the animals employed for these studies had their hepatic DNA prelabeled with BdUrd-^{14}C during liver regeneration prior to the start of these experiments, and to monitor the DNA synthesis that was occurring at the time of the experiment all of the animals received an i.p. injection of dThd-^{3}H (0.5 mCi, 0.05 μmoles/kg) 1 hr prior to sacrifice. The distribution in alkaline CsCl density gradients of hepatic DNA obtained from rats maintained on the control diet is shown in Fig. 1. There was an almost complete separation between the heavy BdUrd-^{14}C-containing segments and the newly synthesized light strands as indicated by dThd-^{3}H incorporation. The fact that BdUrd-^{14}H was incorporated during liver regeneration in these current experiments probably explains the more complete separation between light and heavy Bd-Urd-containing strands reported here as compared to that previously reported for BdUrd-containing rat liver DNA (14). However, when the animals are placed on the carcinogenic diet and sacrificed at 7:30 pm (3 hr after withdrawal of food) of the second day on this diet a significant amount of ^{3}H is associated with the heavy DNA strands (Fig. 1), indicating that some non-semiconservative DNA synthesis had occurred. When the animals were sacrificed at 8:30 am (16 hr after the withdrawal of food) at the end of the second day on the carcinogenic diet the amount of ^{3}H associated with the heavy DNA segments is reduced (Fig. 1), though the degree of non-semiconservative synthesis is still detectable.

DISCUSSION

The DNA excision repair process, first described in bacteria (15), is believed to involve the formation of an initial single strand break near the lesion in DNA; the removal of the modified nucleotides (along with some adjacent nucleotides (2, 3)), use of the information on the complementary strand to resynthesize the excised region; and the joining of the newly synthesized segment to the pre-existing DNA. In the

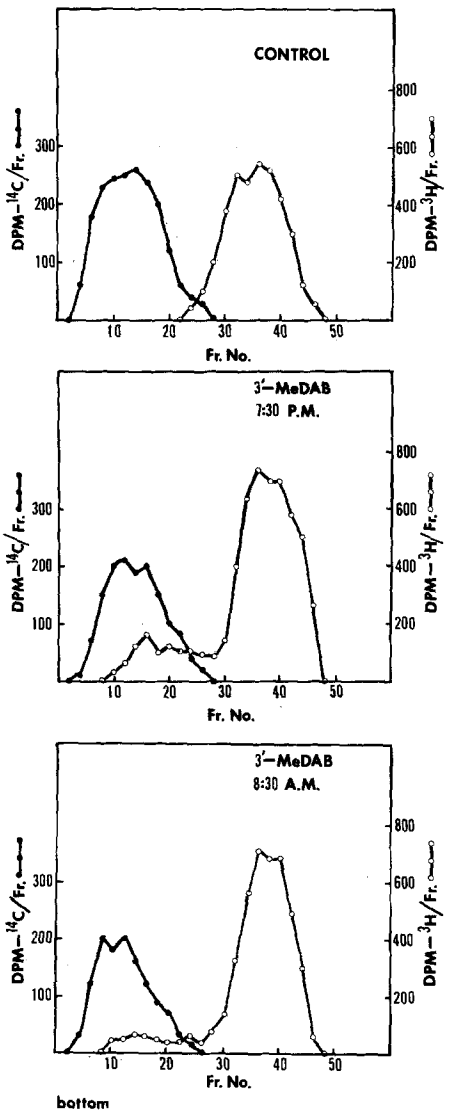

Fig. 1.: Alkaline CsCl density equilibrium centrifugation of rat liver DNA. Hepatic DNA was prelabeled with BdUrd-^{14}C and all animals received an i.p. injection of dThd-^{3}H 1 hr prior to sacrifice. DNA was isolated from control animals and those fed 3'-MeDAB and killed at either 7:30 p.m. (3 hr after withdrawal of food) or at 8:30 a.m. (16 hr after withdrawal of food) of the second day on the carcinogen-contanining diet. Each gradient contained 150 to 250 ug of DNA and was adjusted to an initial density of 1.70 to 1.71. Density equiilbration was obtained by centrifuging at 35,000 RPM (87,000 x g) in a type 40:3 Spinco rotor for 48 hr at 25°. Two drop fractions were collected from the bottom of the gradient and analyzed for radioactivity.

of non-seminconservative DNA synthesis, the distribution of DNA in alkaline CsCl gradients returns to that seen in the control animals (Fig. 1). Analysis by velocity sedimentation in sucrose gradients has shown (16) that after a single injection of the hepatic carcinogen 1-nitroso-5,6-dihydrouracil liver DNA repair synthesis was complete in 4 hr.

These studies indicate that induction of hepatic DNA repair synthesis is an early consequence of 3'-MeDAB ingestion and that the amount of DNA repair occurring at a given time is dependent, in part, on the animals feeding schedule. Shortly after the end of a feeding period the amount of DNA repair synthesis will be high and this will decrease during fasting periods as repair nears completion and no more carcinogen reaches the liver. Therefore, the use of controlled feeding and lighting schedules, as advocated by Potter (11, 12), is highly desirable in studies on animals on carciongen-containing diets.

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REFERENCES

1. Cleaver, J.E., Thomas, G.H., Trosko, J.E., and Lett, J.T., Exptl. Cell Res., 74: 67 (1972).
2. Edenberg, H. and Hanawalt, P., Biochim. Biophys. Acta, 272: 361 (1972).
3. Painter, R.B. and Young, B.R., Mutation Res., 14: 225 (1972).
4. Lieberman, M.W., Baney, R.N., Lee, R.E., Sell, S., and Farber, E., Cancer Res., 31: 1297 (1971).
5. Stich, H.F., San, R.H.C., and Kawazoe, Y., Nature, 229: 416 (1971).
6. Cleaver, J.E., Cancer Res., 33: 362 (1973).
7. Goodman, J.I., and Potter, V.R., Cancer Res., 32: 766 (1972).
8. Cox, R., Damjanov, I., Abanobi, S.E., and Sarma, D.S.R., Cancer Res., 33: 2114 (1973).
9. Damjanov, I., Cox, R., Sarma, D.S.R., and Farber, E., Cancer Res., 33: 2122 (1973).

current studies, repair replication is operationally defined as the incorporation of dThd-^{3}H into segments of parental DNA that have been density labeled with BdUrd-^{14}C. This is the reverse of the labeling procedures employed to measure non-semiconservative DNA synthesis in mammalian cells in culture following exposure to X-irridation (3) or ultraviolet-irradiation (2). However, this was necessary due to the problems associated with attempts at measuring DNA repair synthesis in vivo.

In a previous report (7), employing alkaline sucrose gradient centrifugation for an assessment of DNA repair, it was shown that in rats on a 3'-MeDAB diet, and maintained on a controlled feeding and lighting schedule, hepatic DNA repair synthesis reached a maximum 2.5 hr after the withdrawal of food and was not detectable by 15.5 hr after withdrawal of food (just prior to the start of the next feeding period). These observations have been confirmed in this current study, which was performed during the second day of carcinogen feeding. This early time period was chosen because of our interest in initial molecular events associated with carcinogen exposure, and because after a couple of weeks on the 3'-MeDAB diet the loss of prelabeled DNA would have been so extensive (7) as to have made these studies not possible. DNA repair synthesis was not detectable in animals on the control diet (Fig. 1) and these results were not influenced by the time of day the experiments were performed. When animals were placed on the carcinogenic diet, DNA repair synthesis reached a maximum at 7:30 p.m. (3 hr after the withdrawal of food) (Fig. 1) and was markedly reduced at 3:30 a.m. (16 hr after withdrawal of food) (Fig. 1). The procedure used in this study for the estimation of DNA repair synthesis is more sensitive than that previously employed (7) and this is the probable reason that we are now able to detect a low level of DNA repair synthesis in the livers of animals on the 3'-MeDAB diet examined at 16 hr after the withdrawal of food. If the animals are taken off the carcinogenic diet at the end of the second day and placed on the control diet for 24 hr (40 hr from the end of the last carcinogen feeding period) there is no detectable level

10. Farber, E., Cancer Res., 16: 142 (1956).
11. Potter, V.R., Baril, E.F., Watanabe, M., and Whittle, E.D., Federation Proc., 27: 1238 (1968).
12. Watanabe, M., Potter, V.R., and Pitot, H.C., J. Nutr., 95: 207 (1968).
13. Barbiroli, B., and Potter, V.R., Science, 172: 738 (1971).
14. Jackson, C.D., and Irving, C.C., Chem.-Biol. Interactions, 2: 261 (1970).
15. Pettijohn, D., and Hanawalt, P., J. Mol. Biol., 9: 395 (1964).
16. Stewart, B.W., Farber, E., and Mirvish, S.S., Biochem. Biophys. Res. Comm., 53: 773 (1973).