are necessary to activate opiate processes, 60 to 80 inescapable shocks should be required to produce sensitization to morphine. To test this hypothesis, we gave groups of 20 rats 0, 40, or 80 shocks and, 24 hours later, administered morphine (2 mg/kg) to half of the rats in each group and saline to the other half. Thirty minutes later the rats were given three tail-flick latency tests.

Only the rats that had been given 80 inescapable shocks were hyperreactive to morphine (Fig. 2B). A 2 × 3 analysis of variance revealed that the increase in analgesia observed depended on whether the animal had received morphine and on the number of shocks given ($P < .005$). Newman-Keuls post hoc comparisons revealed that the response of the group receiving 80 shocks and morphine differed significantly from that of all the other groups. Prolonged exposure to inescapable shock evidently elicits hyperreactivity in a system responsive to the opiates. Thus long-term SIA may occur because the system acted on by the endogenous opiates has been made hypersensitive, not because more of the ligand is released during reinstatement of analgesia.

We have demonstrated that both opiate and nonopiate forms of SIA exist and can be produced with the same stressor. This was suggested by Lewis *et al.* (*5*), but they compared continuous with intermittent shock. Our procedure entailed presentation of only one pattern of shock; therefore our findings suggest that a critical determinant of the form of SIA is the number of shocks or the duration of exposure. Prolonged exposure could be important because it allows the animal to learn that it is helpless, or perhaps because it simply provides more stress. Moreover, we have shown that the activation of opiate systems is necessary and sufficient to produce long-term SIA and that opiates and inescapable shock share some common action. This commonality appears to reside in a facilitation of the effectiveness of endogenous opiates rather than in facilitation of their release.

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14. We thank D. R. Thomas, J. W. Rudy, T. Moye, J. Rodgers, R. Drugan, and L. Ashcraft for their helpful advice. Supported by NSF grant BNS 78-00508 to S.F.M. and by ONR grant SRO-001 to J.D.B.

1 May 1981; revised 7 July 1981

Carcinogen-DNA Adducts in Mutagenesis Assays

Bigger *et al.* (*1*) state that the use of liver homogenates in conjunction with the *Salmonella* mutagenesis assay, developed by Ames and co-workers (*2*), as an in vitro screening test for carcinogens can yield misleading results. Bigger *et al.* studied a single carcinogen 7,12-dimethylbenz[*a*]anthracene (DMBA) and found that metabolic activation and the formation of DNA adducts mediated by a rat liver homogenate (S9) system differed from results obtained in intact mammalian cell systems. However, they did not study DMBA adducts bound to the DNA of intact *S. typhimurium*, but instead substituted naked DNA. For the S9 and microsome assay system, a 2-hour incubation was used, but for the intact mammalian cells the exposure time was 24 hours. Thus it is possible that in the mammalian cells certain DMBA-DNA adducts were removed by DNA excision repair during the 24-hour period. In the studies with the S9 fraction the concentration of DMBA was many times higher than in those with the intact cell systems, and much higher than the concentration used in the Ames pour plate assay. Presumably, because of these differences, the amount of DMBA bound to DNA was three to five times higher in assays with the S9 fraction or microsomes than amounts obtained with intact cells. Furthermore, the precise structures of the adducts formed under the various conditions are not known with certainty, nor is it known which of these adducts plays a central role in the carcinogenic process.

Under appropriate conditions of incubation of the parent carcinogen benzo[*a*]pyrene with an S9 activation system, we detected a guanine adduct in the *S. typhimurium* DNA that is identical in chemical structure and stereochemistry to the major benzo[*a*]pyrene diol epoxide adduct found in intact rodent or human cells incubated with benzo[*a*]pyrene (*3*). Stark *et al.* (*4*) found that when *S. typhimurium* was incubated in an S9 assay system with aflatoxin B_1, the major DNA adduct formed was the same as that found in the liver DNA of rats exposed to this carcinogen. In addition, the *S. typhimurium* S9 assay system has proved useful in the screening of a wide range of potential carcinogens (*5*). We would agree that because of marked variations between assay systems, interspecies and intertissue variations, and the multistep nature of the carcinogenic process considerable caution must be exercised in making precise extrapolations from various in vitro systems to the intact organism. It seems likely that the *S. typhimurium* S9 assay system may give more reliable results with some carcinogens than with others. This assay system and assays that employ intact cells for metabolic activation require further study.

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9 September 1980; revised 26 January 1981

We initiated comparative studies of carcinogen metabolism in different systems to examine the assumption that the subcellular Aroclor-induced rat liver metabolizing system, recommended by Ames *et al.* (*1*) for general mutagenesis

0036-8075/81/0918-1411$01.00/0

screening, effectively duplicated the activation of carcinogens in target tissues in vivo. In a series of studies (*2, 3*), we have demonstrated that the DNA reactive metabolites of 7,12-dimethylbenz[*a*]anthracene (DMBA) generated by Aroclor-induced rat liver microsomes or S9 fraction differ substantially from those generated in intact cellular systems in vitro and a target tissue, mouse skin, in vivo. The most dramatic difference between intact cellular systems and the subcellular systems is that in the latter the preponderance of individual reactive metabolites varies with the DMBA concentration, thus weakening confidence in the interpretation of mutagenesis dose-response data (*3*). We have not suggested nor intentionally implied that the subcellular system is, therefore, a poor model for metabolic activation for all carcinogens, but we emphasized that, in the light of our findings, it would be unwise to assume a priori that the subcellular system is an adequate model for the activation of all carcinogens.

Neither the aflatoxin B_1 study (*4*) nor the benzo[*a*]pyrene study (*5*) discussed by Grunberger *et al.*, report DNA adduct data for the Aroclor-induced rat liver system. The aflatoxin B_1 data does show, however, that at two substrate concentrations a phenobarbital-induced rat liver fraction accurately reproduces the in vivo one-step metabolic activation of this carcinogen. In the benzo[*a*]pyrene study, mouse liver S9 and a single substrate concentration were used. This carcinogen, like DMBA, exhibits concentration-dependent shifts in metabolite profile in subcellular systems (*6*), requires three sequential metabolic reactions for activation to the carcinogenic metabolite, and contains alternative sites for metabolism to DNA reactive species. A dose-response study with the mouse liver system is required, therefore, for a full evaluation.

We agree that the specific DNA adducts responsible for carcinogenesis are not known with certainty for any carcinogen, but there is evidence to show that specific DNA reactive metabolites play a central role in carcinogenesis (*7*). We have used DNA adduct analysis merely to monitor the generation of these specific metabolites. The excision repair argument of Grunberger *et al.* is not substantiated by experiment because DMBA-DNA adducts have been found to be excised exceptionally slowly in the mouse embryo cell system (*8*). Our reports on dose response (*3*) show that the criticism that our findings are based on comparisons at different binding levels is unfounded. The use of naked DNA rather than *Salmonella* also does not compromise our findings. Presumably, the implied criticism is that the *Salmonella* membrane and the nuclear membrane in mammalian cells filters out all but the specific DNA reactive metabolites involved in carcinogenesis. However, it has been shown, for example, by Baird *et al.* (*9*), that the DNA of mammalian cells exposed to the K-region epoxide of 7-methylbenz[*a*]anthracene contained the same products as naked calf thymus DNA exposed to this epoxide; and Santella *et al.* (*5*) state that they obtained the same products with naked DNA and intact *Salmonella* but clearly got different products in *Salmonella* DNA when differently induced mouse liver S9 was used.

Overall, the conclusions of Grunberger *et al.* do not differ substantially from ours. However, they emphasize that "under appropriate conditions" some liver homogenates may accurately model in vivo activation. Because of the wide application of these in vitro systems in the detection of potential carcinogens whose structures, pathways of metabolism, and mechanism of action are unknown, we reemphasize, on the basis of our findings, that the accurate reproduction of in vivo activation cannot be assumed.

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26 June 1981