Mutagenicity of Primary and Secondary Carcinogens Altered by Normal and Induced Hepatic Microsomes[1] (37103)

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To establish the role of the activity of the microsomal biotransformation system in chemical carcinogenesis, the mutagenic effect of primary (ultimate) and secondary (potential) carcinogens after exposure to isolated hepatic microsomes was studied. The principal enzyme system, suggested to be active in the metabolism of carcinogenic compounds, is the nonspecific cytochrome P-450 dependent mixed-function oxidase (1). This microsomal enzyme system is inducible by many substrates, including some environmental contaminants (2). The involvement of the microsomal enzymes in carcinogenesis is suggested by the observed increased carcinogen biotransformation in animals (3) and isolated cells (4) following exposure to inducing substrates. Most carcinogenic compounds are mutagenic for microorganisms (5). The primary are themselves mutagenic, the secondary are inactive until metabolized to a mutagenic form (6). Metabolism of primary carcinogens may reduce mutagenic and carcinogenic properties. Both types of metabolic conversion, activation and deactivation, have been studied by host-mediated assay, which tests the ability of laboratory animals to alter the mutagenicity of carcinogens (6). Similar activity also has been demonstrated in the 30,000*g* supernatant of mice liver homogenates (7).

To demonstrate the precise relationship between the activity of the microsomal enzyme system and the biological activity of carcinogens, an assay is required in which both activities can be measured. For this purpose the ability of isolated normal or induced microsomes to alter the mutagenic activity of two carcinogens, dimethylnitrosamine (DMN) (8) and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) (9) was studied. These compounds were chosen as examples of primary (MNNG) and secondary (DMN) carcinogens.

Methods. Hepatic microsomes were isolated from male Swiss–Webster mice untreated or pretreated four days before sacrifice from a single ip injection of 500 mg/kg of the polychlorinated biphenyls (PCB), Arochlor 1254, an inducer of the microsomal enzyme system (10) and a wide-spread polluting agent (11). The procedures to prepare the microsomes and to determine the concentrations of microsomal protein and cytochrome P-450 and the activity of aminopyrine demethylation have been described (2). The mutagenicity of DMN and MNNG was assayed by a bacterial auxotroph reversion test, similar to that described by Ames (12). Bacterial cells were centrifuged from an exponentially growing culture of *Bacillus subtilis* 168 *ilv* which requires isoleucine and valine for growth, and resuspended in an incubation mixture containing microsomes and a NADPH generating system. Mutagen was added and the mixture incubated with shaking at 37°. Samples were withdrawn at different times and plated for total colony forming units (cfu) and *ilv*$^+$ cfu on appropriate selective media (13).

Results. Microsomes prepared from PCB treated mice had 2–3 times as much cytochrome P-450 compared to normal ones and corresponding increases in the activity of

[1] Supported by grants AM 03846 U.S. Public Health Service, National Institutes of Health, and VC-38 from the American Cancer Society, and Deutsche Forschungsgemeinschaft.

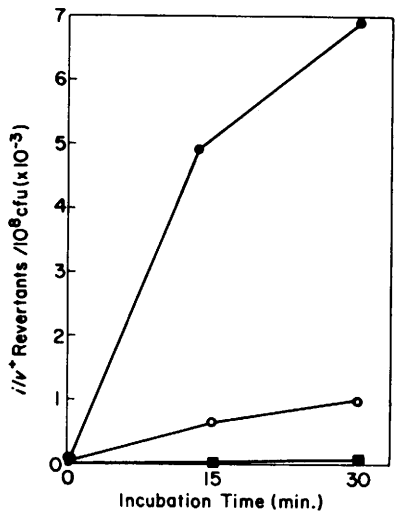

FIG. 1. Frequency of DMN *ilv*$^+$ revertants of *B. subtilis* 168 *ilv*. The complete mixture contained 200 m*M* DMN, 10 m*M* $MgCl_2$, 50 m*M* DL-isocitrate-Na_2, 5 m*M* NADP, 0.2 mg isocitrate dehydrogenase, 15 mg microsomal protein and 3–7 × 10^8 bacterial cfu in 2.5 ml 0.1 *M* phosphate buffer at pH 7.4 and was incubated at 37°. In control mixtures (■—■) either microsomes or the NADPH generating system were omitted. The system with normal microsomes (○—○) contained 0.7 nmole P-450/mg microsomal protein, that with PCB induced microsomes (●—●) 2.5 nmole P-450/mg microsomal protein. The activity of aminopyrine demethylation expressed as nmole HCOH formed/mg protein/min was 10.2 and 25.4 for normal and PCB induced microsomes, respectively.

cytochrome P-450 dependent aminopyrine demethylation (see legends to Figs. 1 and 2). DMN added directly to cultures of *B. subtilis* was not mutagenic at concentrations as high as 300 m*M*. It was converted, however, to a mutagen when exposed to microsomes in the presence of a NADPH generating system (Fig. 1). Microsomes from PCB treated mice were more effective in the activation of DMN than those from untreated mice.

Suggestive evidence for MNNG inactivation has been obtained with the host-mediated assay (6). When MNNG was incubated with isolated microsomes, its mutagenic activity was reduced but microsomes from untreated mice inactivated less mutagen than microsomes from PCB-treated ones (Fig. 2).

Discussion. The *in vitro* system described here provides a rapid assay suitable for correlating microsomal enzyme levels with the activation and inactivation of the mutagenicity of carcinogens. The ability of isolated microsomes to modify the biological activity of mutagens confirms the suggestion that the microsomal biotransformation system plays a key role in chemical mutagenesis. Although there is probably a relationship between carcinogenesis and mutagenesis, there are still differences which have not been explained. The observation that induced microsomes inactivate the mutagenic activity of primary carcinogens or activate that of secondary ones to a greater extent than normal ones suggests that the status of microsomal metabolism affects the response of an individual to carcinogen exposure. If we are permitted to extrapolate from mutagenesis to carcinogenesis, we might understand some old observations. Differences in the status of the biotransformation system at the time of

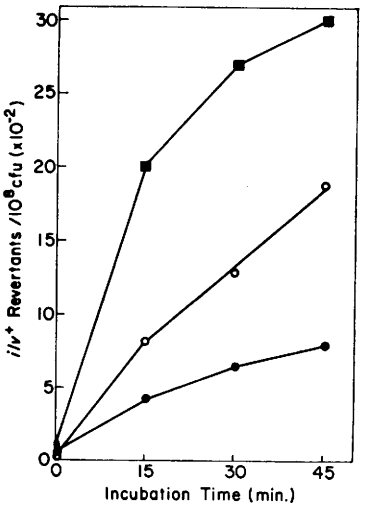

FIG. 2. Frequency of MNNG-induced *ilv*$^+$ revertants of *B. subtilis* 168 *ilv*. The complete incubation mixture contained 0.03 m*M* MNNG and 3 mg microsomal protein. All other conditions, including the controls (■—■) were as in Fig. 1. The system with normal microsomes (○—○) contained 0.7 nmole P-450/mg microsomal protein, that with PCB induced microsomes (●—●) 1.5 nmole P-450/mg microsomal protein. The activity of aminopyrine demethylation expressed as nmoles HCOH formed/mg protein/min was 10.3 and 16.7 for normal and PCB induced microsomes, respectively.

exposure may explain why only a limited percentage of exposed individuals develop cancer. Furthermore, nutritional disturbances, particularly protein (14) or riboflavin (15) deficiency, which reduce microsomal biotransformation, may explain the geographic prevalence of certain cancers such as those of the liver (16), because malnourished populations should be more susceptible to primary carcinogens. By contrast, in the well-nourished technologic societies, the biotransformation system induced by chemical pollutants, drugs and pesticides may prove to be the greater hazard.

The authors acknowledge the technical assistance of Mr. Appelt.

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Received Oct. 17, 1972. P.S.E.B.M., 1973, Vol. 142.