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DIFFERENCES IN BENZO(a)PYRENE METABOLISM BETWEEN LUNG AND LIVER HOMOGENATES

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Although recently accumulated data on the metabolism of benzo(a)pyrene (B(a)P) in liver homogenates or in microsomes have shed light on the complicated pathways of B(a)P, an important problem remains whether the metabolic pattern of B(a)P in the target organ is the same as in the liver. In parallel experiments where rat-liver and rat-lung preparations were used, Grover and Sims[1] and Grover[2] showed that metabolic patterns in both tissues are quantitatively different, but not qualitatively different. In their experiments, ^{3}H-labelled B(a)P was used and hydroxy, epoxide and diol metabolites were estimated by radioactivity. By using the fluorometric method and electron spin resonance (ESR) method, we have carried out a comparative study on the metabolism of B(a)P in rat-liver and lung homogenates, and different metabolic patterns between two tissues were observed, which will be reported in this communication.

Male Sprague-Dawley rats, 5 weeks old (100-130 g) were killed 24 hr after the i.p. administration of 3-methylcholanthrene (MC) with a single dose of 20 mg/kg dissolved in corn oil once a day for 3 days or phenobarbital (PB) with 60 mg/kg in saline once a day for 3 days. Lungs were homogenized in 2 volumes of 0.05 M Tris-HCl buffer (pH 7.5) containing 0.15 M KCl in a Potter-Elvehjem homogenizer and livers were homogenized in 3 volumes of the same buffer. The homogenates were centrifuged at 9000 x g for 20 min and the supernatant was used to study the metabolism of B(a)P.

The reaction mixture, in a total volume of 100 ml contained 80 μmole of NADPH, 5 mmole of Tris-HCl buffer (pH 7.5), 15.4 mmole of KCl, 10 μmole of B(a)P in 4 ml of methanol and 60 ml of the supernatant fraction. After incubation at 37° for 10 min, 10 ml of reaction mixture was used for the fluorometric assay, which is the same as the method of Nebert and Gelboin[3], except that the hexane extract was evaporated to dryness to redissolve in 1 ml of hexane. The remaining mixture for the ESR measurement was extensively extracted with benzene (90, 90 and 90 ml) and the extract was evaporated in vacuum by tap water for overnight. The evaporated residue redissolved in 0.3 ml benzene was subjected to the ESR measurement.

In Fig. 1, the excitation spectra for lung homogenates were given in comparison with those for liver homogenates. The difference in spectral pattern is apparent; thus, the maximum peaks around 320 nm and 460 nm were shifted to the longer wavelength from (a) to (f). The most

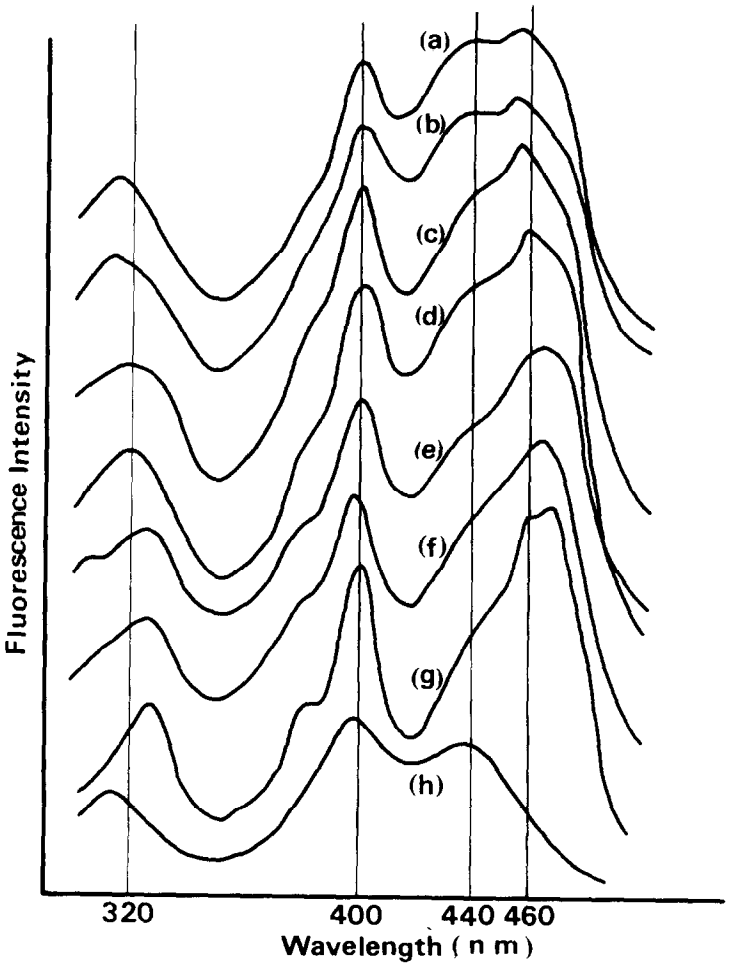

Fig. 1. Excitation spectra of alkali-extractable products formed by lung homogenates from control (a), PB-treated (b), and MC-treated rats (c); by liver homogenates from control (d), PB-treated (e), and MC-treated rats (f); (g) and (h) are the spectra for the authentic samples of 3-OH-B(a)P and 9-OH-B(a)P, respectively. No difference was observed in the emission maxima for all spectra, from (a) to (f). Induction ratio by MC was 10-15 in lung and liver and by PB it was 4-5 in liver but no induction was observed in lung.

remarkable fact is that the maximum peak appears at 440 nm in lung homogenates from control and PB-treated rats (a and b), but it is absent in liver homogenates from PB- and MC-treated rats (e and f). While the latter spectra closely resemble to that of authentic 3-OH-B(a)P, the component at 440 nm in the former spectra is not considered to be originated from it. The most likely candidate for this component is 9-OH-B(a)P which has the excitation maximum at 400 nm and 440 nm. In fact, the similar spectrum of B(a)P metabolites as in Fig. 1 (a) was reported in the reconstituted liver microsome system and the analysis revealed that the metabolites contained 9-OH-B(a)P half as much as 3-OH-B(a)P[4]. In our case, the participation

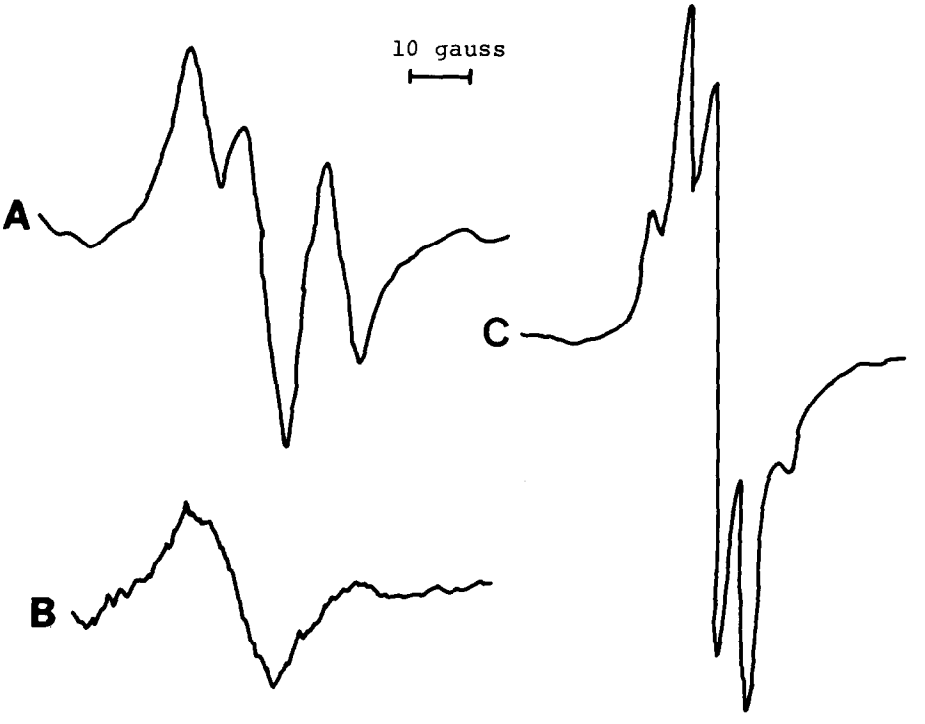

Fig. 2. ESR signals of the free radicals produced by incubating B(a)P with lung homogenates from control (A), PB-treated (B), and MC-treated rats (C). Signal C is reduced to 1/5 of the original one. Modulation width: 4 gauss.

of other phenolic metabolites cannot be excluded. In order to characterize the metabolites in lung homogenates, more detailed investigation using high pressure chromatography is required. However, it is worth noticing that the different metabolic pattern of lung and liver homogenates could be detected by the usual fluorometric method.

By using the ESR method, we previously found that 6-oxybenzo(a)pyrene (6-oxy-B(a)P) radical was formed as a metabolite by incubating B(a)P with liver homogenates from control, PB-treated and MC-treated rats[5,6], and this result was confirmed by Lesko et al[7]. We applied the same technique to the case of lung homogenates and found that the feature of metabolism of B(a)P in lung homogenates was different from the case of liver homogenates. Thus, the signals were different each other in their linewidth and signal pattern when lung homogenates from the control, PB-treated and MC-treated rats were used (Fig. 2), indicating that different species of free radical are formed metabolically from B(a)P in lung homogenates from the control, PB-treated and MC-treated rats. Magnitudes of the signals obtained by incubating B(a)P with homogenates from control and PB-treated rats were comparable, but in the case of

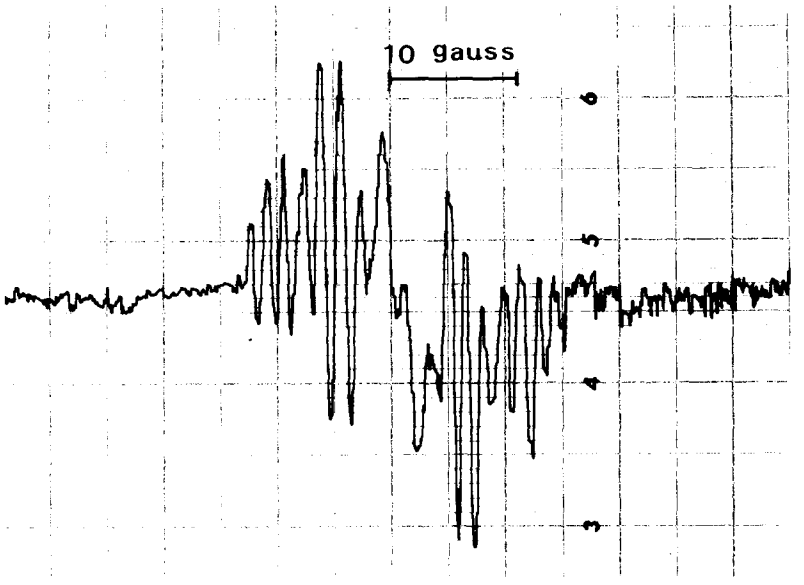

Fig. 3. Hyperfine structure of the signal (C) in Fig. 2 after degassing the sample tube. Modulation width: 0.8 gauss.

homogenates from MC-treated rats, the magnitude of the signal was several times as the former ones. After degassing, the signal (C) in Fig. 2 was further resolved into hyperfine structure as seen in Fig. 3, which was previously identified as 6-oxy-B(a)P radical in liver homogenates from control, PB-treated and MC-treated rats[5,7]. Identification of the signals (A) and (B) in Fig. 2 is not easy because of difficult resolution of these signals into hyperfine structure. However, from the fluorometric data indicated in Fig. 1, free radical derived from 9-OH-B(a)P is considered to be a plausible candidate for the signal (A). Confirmation of this supposition is under progress in our laboratory.

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