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Detection of Mutagenic Activity in Cigarette Smoke Condensates

(carcinogenesis/*Salmonella* tester strains/microsomal activation)

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ABSTRACT The *Salmonella typhimurium* microsomal test system for mutagenic activity was successfully used to detect the presence of mutagenic compounds in the smoke condensates of several types of cigarettes. The condensates were shown to contain compounds which could cause frameshift mutations when activated by microsomal enzymes. An analysis of fractions of smoke condensate revealed that the detected mutagenic activity was distributed in several of the fractions. Most of the activity of the whole condensate was in basic fractions and in a weakly acidic fraction. Condensates from cigarettes treated with magnesium nitrate differed from other condensates in two respects. They contained frameshift mutagens which did not require microsomal activation and mutagens which could cause base-pair substitution mutations. Although the detection system usually employs rat liver microsomal preparations, a rat lung microsomal preparation was also found to be capable of converting smoke condensates and known chemical carcinogens into mutagenic forms.

In this laboratory a simple, quantitative, and sensitive bacterial assay system for the detection of mutagenic compounds has been developed (1–5). The system determines the ability of compounds to revert histidine auxotrophs of *Salmonella typhimurium* to histidine prototrophy, an event which requires mutation of the DNA. The use of a rat liver microsomal fraction in the assay system extends the scope of the system to include compounds which require nonbacterial metabolism for conversion into active mutagenic agents (6). A wide variety of carcinogens have been shown to be mutagens with this test system (1–6). This, and other work, showing that chemical carcinogens are mutagens strongly supports the repeating theory that cancer can be caused by somatic mutations (6). The economy of this bacterial assay suggests its usefulness as a tool in rapidly obtaining information about the potential mutagenic/carcinogenic activity of uncharacterized compounds in complex mixtures.

We have employed the *Salmonella* test system in characterizing cigarette smoke condensate, i.e., the particulate matter of cigarette smoke which contains over 1200 known components and many as yet unidentified compounds (reviewed in ref. 7). Cigarette smoke condensate is known to act as a carcinogen and cocarcinogen in mouse skin (refs. 8, 9), and statistical evidence indicates that cigarette smoke is a contributing factor in the cause of human cancer (10–12). We were able to demonstrate that cigarette smoke condensates have mutagenic activity. The assay system enabled us to assess the type of mutagenic activity present and the role of mammalian metabolism in the expression of this mutagenic activity. We have also examined fractions of the cigarette smoke condensate to determine the distribution of the detected mutagenic activity among the classes of chemical compounds which are separated by the fractionation procedure.

MATERIALS AND METHODS

Bacterial Strains. The *Salmonella typhimurium* strains used, TA1535, TA1536, TA1537, and TA1538, have been described in detail previously (5).

Compounds. Glucose-6-phosphate and NADP were obtained from Sigma. Benzo[*a*]pyrene and 2-acetylaminofluorene were from Aldrich. Schuchardt (Munich) was the source of 2-aminoanthracene and 2-aminofluorene. Aroclor 1254 (Monsanto) was a gift of Alan Poland (Department of Pharmacology and Toxicology, University of Rochester) and is commercially available from Analabs, North Haven, Conn.

Cigarette Smoke Condensates. Crude cigarette smoke condensates from control cigarettes, high-charcoal filtered cigarettes, and nitrate-treated cigarettes were supplied by Philip Thayer (Arthur D. Little, Inc.) who furnished the following description. Each condensate was made in a small batch from commercial cigarettes with a standard tobacco blend. The high-charcoal cigarettes were attached to an experimental filter holder containing 300 mg of activated charcoal. The nitrate-treated cigarettes contained 10% $Mg(NO_3)_2$. The control and nitrate-treated cigarettes had no filter. The cigarettes were smoked on a standard smoking machine, taking 35-ml puffs of 2-sec duration once per min. The smoke was condensed in a trap cooled with liquid air. The collected material was thawed, suspended in acetone, the acetone removed, and samples dissolved in dimethyl sulfoxide. The samples contained 0.26 g/ml, 0.076 g/ml, and 0.128 g/ml for the control, high-charcoal filtered, and nitrate-treated cigarettes, respectively. These values represent the equivalent of 10, 5.2, and 8 cigarettes worth of condensate per ml.

Crude condensate, fractions, and a reconstituted sample (all fractions combined in the appropriate amounts) from University of Kentucky 1A1 low nicotine cigarettes were provided by Richard Kouri (Microbiological Associates, Inc.) who furnished the following description. Crude cigarette smoke condensate was fractionated according to the procedure of Swain *et al.* (13). Detailed procedures for producing the smoke condensate from the 1A1 cigarette and analyses of the fractions are published (14). The samples used in these assays are from lot number 2 of the 1A1 cigarette smoke condensate. Samples, dissolved in dimethyl sulfoxide to a concentration of 1 mg/ml, were shipped in dry ice/acetone and stored at −80°.