MUTAGENICITY TESTING OF COMPLEX MIXTURES DERIVED FROM HUMAN BODY FLUIDS

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Although short-term bioassays have usually been developed by testing individual chemicals, samples which need to be evaluated for potential human health hazard are often complex mixtures. These mixtures may contain many toxic substances, each present in small quantities. To detect such toxins in bioassays, sample preparation will involve concentration of the compounds of interest and removal of material which will interfere with the test system. If the toxicity is due to many compounds, isolation and concentration of the total biological activity will be useful either as a preliminary step for chemical analysis or as a method for characterizing some of the toxic and chemical properties of the mixture. Mutagenicity tests are useful for monitoring genotoxic activity during such isolation procedures, since these assays are relatively quick and simple. Moreover, a large number of carcinogens are mutagenic (McCann et al., 1975; Rinkus and Legator, 1979); therefore, isolating mutagenic activity may develop procedures which will be useful in isolating carcinogens. Using simple chemical separation techniques and mutagenicity testing, we have highly concentrated the mutagens in smokers' urine and have elucidated some of their chemical characteristics.

We elected to study smokers' urine for several reasons. First, smokers' urine had already been shown to be mutagenic in the Salmonella assay system by Yamasaki and Ames (1977). Therefore, an exposed population that was positive and a control...
population that was negative were available for developing preparative and analytical procedures for assaying urine samples. Second, urine is one of the more easily sampled body fluids. Third, characterization of the mutagens in urine due to smoking might result in criteria that would allow these mutagens to be distinguished from those due to other exposures. Finally, smokers have been observed to have an elevated incidence of bladder cancer (USDHEW, 1979). Although the correlation of mutagenic urine with bladder cancer is not known, our studies might help to evaluate the contribution of mutagens in urine to the risk of developing bladder cancer.

Yamasaki and Ames (1977) showed that XAD-2 resin could be used to concentrate the mutagenic activity in smokers' urine and to separate the mutagens from histidine present in the urine. Our protocol for characterizing the mutagens in smokers' urine employed standard chemical separation procedures subsequent to XAD-2 extraction (Figure 1). Details of this protocol have been published (Putzrath et al., 1981).

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**Figure 1.** Preparation of urine extracts for mutagenicity testing.