PROJECT TITLE: Toxicity of Inhaled Mycotoxins

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This project was originally planned to include two major areas of study: 1) To develop a rapid, sensitive radioimmunoassay for a specific group of mycotoxins to be used in animal exposure studies to quantify mycotoxin concentrations in experimental aerosol clouds and 2) to study the histopathological effects of various mycotoxins in experimental animals via the pulmonary route. An immunochemical method for measuring the mycotoxins in question was developed at the University of Wisconsin during the course of our work and for this reason and because we do not yet possess aerosol exposure facilities which provide the necessary safety to laboratory personnel in handling carcinogenic substances, we decided to abandon our plans to expose animals by aerosol. Therefore, rats were exposed by intratracheal instillation.

Our histopathological work was hampered to a degree by insufficient manpower in the Pathology Section, resulting in excessively long tissue processing times and also by the earlier policy of maintaining an in-house rat breeding colony. Fortunately, both of these problems have been addressed and we have completed the experimental portion of a study of secalonic acid D (SAD). Not all of the tissues have been examined as yet but it is clear that SAD is toxic to rat lung tissue at much lower doses than previously reported. Animals were exposed both via the trachea and the esophagus but at the doses used, pathologic changes were confined to the lungs and were observed only in the intratracheal group. The changes included acute inflammatory cell reaction at the alveolar duct level, necrosis of the bronchial epithelium, focal areas of granulomatous inflammation, acute inflammatory changes in the terminal bronchioles and hyperplasia of the epithelium. There was no evidence of dysplasia or neoplasia. A manuscript is being prepared for publication.

Aerosol challenge experiments with Serratia marcescens were performed to study the effect of T-2 toxin on clearance of bacteria from rat lung. Fisher 344 (SPF) rats were given 0.25 mg/kg of T-2 toxin in Krebs-Ringer solution by intratracheal instillation whereas control animals received the carrier solvent alone. This dose is ca. 1/15 the published LD_{50} oral dose for rats. After 16 hours, the rats were challenged by the bacterial aerosol. Groups of animals were sacrificed by exsanguination immediately after exposure and 4 hours later, the lungs were removed, homogenized in sterile distilled water and 0.1 ml aliquots from serial ten-fold dilutions were plated on agar media. After 4 hours, the control animals had cleared 68% of the bacteria initially present whereas treated animals had reduced the number of viable bacteria by only 45%

We are also investigating the effects of various mycotoxins on rat alveolar macrophages (AM) in vitro. Our preliminary findings have demonstrated that T-2 toxin and diacetoxyscirpenol (DAS) are highly toxic to these cells and have shown that AM cell viability was reduced to $\leq$ 50% at concentrations as low as 20 μM T-2 or DAS. We have observed significant inhibition of the ability of rat AM to phagocytize zymosan particles and intact cells of Saccharomyces cerevisiae by these mycotoxins. These observations represent the foundation for a new project entitled "Effect of Mycotoxins on Pulmonary Macrophages" to begin FY-81.

The best known and most hazardous group of mycotoxins are the aflatoxins and as yet only fragmentary circumstantial evidence is available to suggest that grain workers may be at risk because of exposure to these mycotoxins. We presented two papers at the International Grain Dust Symposium in Saskatoon, Saskatchawan in November, 1977. In this work we demonstrated an extremely high incidence of contamination of various samples of Georgia corn by Aspergillus flavus. When the corn was ground and aerosolized in a sealed

laboratory system, A. flavus represented over 80% of the fungal colonies isolated from Andersen sampler plates. The studies further demonstrated that ca. 25% of the colonies isolated arose from particles less than 5 μm aerodynamic diameter. In addition to A. flavus, several other fungal species were isolated including Aspergillus fumigatus and Aspergillus terreus both of which produce spores <5 μM aerodynamic diameter and which can readily reach the lower alveolar spaces. This work led us to question whether aflatoxin can exist in significant concentrations in airborne grain dust particles.

Thin layer chromatography is the traditional method of aflatoxin analysis but work in recent years has suggested that high performance liquid chromatography (HPLC) may become the method of choice. Unfortunately, each of the currently available HPLC systems for the aflatoxins has serious deficiencies (solution quenching, etc.). We were able, however, to adapt cleanup and analytic procedures to permit detection of amounts as low as 600 pg of aflatoxin B_1. In cooperation with the Environmental Investigations Branch who collected airborne grain dust samples, we have begun examining various samples for the presence of aflatoxins and recently demonstrated the occurrence of significant quantities of aflatoxin B_1 in respirable size particles of corn dust from Georgia. This work has been accepted for publication in the Journal of Toxicology and Environmental Health.

Publications and Completed Manuscripts

1. Sorenson, W. G., M. J. Peach III, J. P. Simpson, S. A. Olenchock and G. Taylor. 1980. Size Range of Viable Fungal Particles from Aflatoxin-Contaminated Corn Aerosol. In: Occupational Pulmonary Disease: Focus

on Grain Dust and Health. Academic Press, N.Y. J. A. Dosman and D. J. Cotton (Eds.). 48:527-536.

2. Peach, M. J. III, S. A. Olenchock, W. G. Sorenson and Pervis C. Major. 1980. Relevance of Grain Dust Collection Technique to Respiratory Disease Studies. In: Occupational Pulmonary Disease: Focus on Grain Dust and Health. 46:507-512.

3. Sorenson, W. G., J. P. Simpson, M. J. Peach III, T. D. Thedell and S. A. Olenchock. 1981. Aflatoxin in respirable Corn Dust Particles. Accepted for publication in J. Toxicol. Environ. Hlth.