VC 7.0-DP/REV-UL

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Mr. Joseph T. Seawell
Program Manager
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RE: 2nd Quarterly Report for the Manufacturing Chemists Association's Agreement with the University of Louisville for the 1979-80 Fiscal Year.

Dear Mr. Seawell:

The following describes what has been completed during the 2nd quarter of the Manufacturing Chemists Association's agreement with the University of Louisville entitled, "Research Techniques and Methods for Detection and Prevention of Carcinogenesis in Industrial Workers". Progress for each technical proposal will be reported separately.

Technical Proposal A -- Immunological Systems for the Detection of Vinyl Chloride and Other Chemical Injury. H.P. Fortwengler

Fortwengler's laboratory has continued evaluating the immune system of industrial workers to determine whether cancer can be detected earlier or to identify those at high risk.

HLA Frequencies in V.C. Workers.

A compilation of HLA frequencies in V.C. workers is continuing to determine if a possible increased association of certain "tissue types" can be determined in individuals with angiosarcoma or other chemically related disease. To date, approximately 494 individuals from the Louisville vinyl chloride polymerization plant have had their lymphocytes isolated and typed for more than 27 different HLA-A and HLA-B antigens.

A Search for Evidence of a VC-Induced Tumor Antigen.

A search for evidence of a VC-Induced tumor antigen has lead to a finding that these tumors have an increased concentration of antigenic coagulation Factor VIII. Factor VIII is a known

1) We have completed data reduction and have a manuscript now under review by all co-authors for January or February, 1980 submission to the Journal of Clinical Pathology relative to the following study. We developed complete urinary GAG excretion profiles from 24 hour urines from 24 vinyl chloride workers, half with and half free of liver disease. Our conclusion is that GAG profiles may reflect active liver disease. A copy of the final manuscript will be submitted with the final report.

2) We completed the laboratory work involved in a repeat of the study we reported in Gastroenterology 73:1229 in 1977. At the end of the quarter, we had not yet started data reduction on this project.

3) We completed an extension and a repeat of the Morris Hepatoma study. We reported in Gastroenterology 75:972, 1978.

In the earlier study, we evaluated three tumor lines; in 1979 we looked at these three plus three more. A paper will be submitted in January for presentation at next May's meeting of the American Federation for Clinical Research in Washington.

These studies address the role of GAGs in the behavior of hepatic malignancy as well as the usefulness.

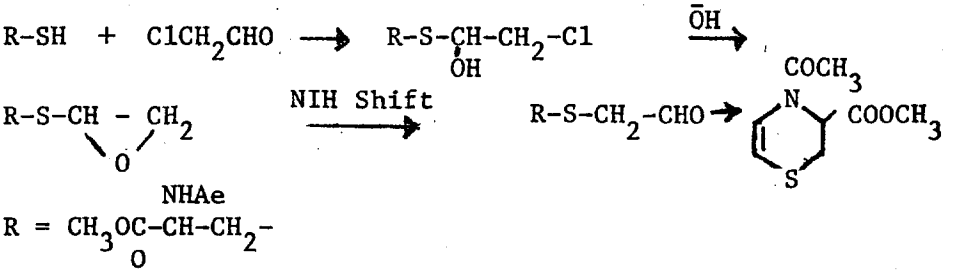
Some recent presentations made as a result of our work are listed below.

Secskas, E., Kupchella, C.E., Kennedy, J. and Espinosa, E. Glycosaminoglycan Changes Associated with Hepatic Tumors: The Contributions of Regeneration and Necrosis. Presented at the Association for American Physicians/ American Society for Clinical Investigation/American Federation for Clinical Research National Meeting, Washington, D.C., May 7, 1979.

Kupchella, C.E., Drake, E. and Secskas, E. Glycosaminoglycan Patterns in Necrotic Liver, Regenerating Liver, and in Three Morris Hepatomas having Different Growth Rates. Presented at the Seventh Bi-Annual Hepatoma Conference, Washington, D.C., May 10, 1979.

elucidate the stepwise formation of the thiazene-N-acetylcysteine methyl ester was allowed to react with chloroacetaldehyde in chloroform at 0°C. The initial product, plausibly the hemithioacetal $H_3CO-\overset{O}{\overset{\|}{C}}-CH-(NHCOCH_3)CH_2S-\underset{OH}{CH}-CH_2Cl$, eliminated HCl upon neutralization with aqueous sodium hydroxide to produce the corresponding epoxide $H_3COCOCH(NHCOCH_3)CH_2S-CH\underset{O}{<}CH_2$. This structural assignment is supported by its pmr spectrum. Furthermore, when this epoxide was extracted into chloroform, it rearranged to the S-acetaldehyde $H_3COCOCH(NHCOCH_3)CH_2-S-CH_2CHO$, identified by its pmr spectrum and comparison with that formed from the reaction of N-acetylcysteine with chlorooxirane.

Even though chlorooxirane and chloroacetaldehyde eventually give the same final product with N-acetylcysteine, the rate of reaction and the intermediates in the two reactions are different. The chlorooxirane conjugates instantaneously with the sulfhydryl compound, while chloroacetaldehyde takes about 2 1/2 hours for a comparable reaction. The reaction pathway for chloroacetaldehyde is proposed as follows.



These two metabolites are therefore both similar and different in their detoxification reaction with the RSH. Further study will continue to unravel the significance of either or both of chlorooxirane and chloroacetaldehyde in the mutagenesis/carcinogenesis problem of vinyl chloride.

Technical Proposal F -- Assays for the Carcinogenic Potential of Industrial Chemicals Utilizing Prokaryotic and Eukaryotic Systems. U.N. Streips in collaboration with G. Sonnenfeld

During the 2nd quarter we have concentrated on developing the interferon induction assay as a valid test for the carcinogenic potential of industrial chemicals. To this end the following results have been obtained:

shown by absence of fluorescence with absorbed antiserum, non-immune serum or buffered saline. With each protein, the fluorescence was restricted to the cell cytoplasm and an intense degree of fluorescence indicated relatively high levels of these proteins in a large proportion of the cells. Other individual cells showed varied degrees of fluorescence suggesting heterogenicity of the cell population with respect to the synthesis of these plasma proteins. Demonstration of presence of these proteins in the tumor cells by immunofluorescence is in agreement with results obtained by immunodiffusion as previously reported and thus provide another approach for the study of serum proteins and possibly other protein antigens by hepatoma.

Technical Proposal I -- Vinyl Chloride Metabolism in Isolated Liver Cells.
Richard C. Feldhoff

As reported for the preceding quarter we have experienced some difficulty in purchasing all of the special supplies needed for the in situ liver perfusion technique using our recently acquired liver perfusion apparatus.

In the meantime, we have continued to produce and purify rabbit anti-rat albumin antiserum. The antibodies which we are purifying will be used to quantitate the effects of ethanol and chemical monomers on the synthesis of serum albumin relative to total protein synthesis. Albumin is a constitutive product of the liver and perturbations in its rate of synthesis reflect alterations in normal cellular metabolic processes. Albumin synthesis has been reported to be particularly influenced by the levels of essential amino acids and ethanol. One of the primary intracellular effects is polysome disaggregation. Techniques are being developed to quantitatively recover undegraded polysomes from detergent-treated post-mitochondrial supernatants.

This completes the quarterly report from the University of Louisville Chemical Monomer Research Group. If there is need for further information or clarification, please do not hesitate to contact me.

Sincerely yours,

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CHT:vb