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Environmental Affairs

To: Vinyl Chloride Project Panel

Subject: Third Quarterly Report Covering Research Performed
Under the Agreement of Fiscal Year 1979-80 on
"Research Techniques and Methods for Detection and
Prevention of Carcinogenesis in Industrial Workers",
University of Louisville.

Gentlemen:

Enclosed is your copy of the subject report.

Progress under each section of the technical proposal is
reported separately.

Sincerely,

A handwritten signature in cursive script, appearing to read "J. T. Seawell".

J. T. Seawell
Project Administrator
Vinyl Chloride

JTS:md
Enclosure

Formerly Manufacturing Chemists Association—Serving the Chemical Industry Since 1872

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Mr. Joseph T. Seawell
Program Manager
Manufacturing Chemists Association
1825 Connecticut Avenue N.W.
Washington, D.C. 20009

RE: 3rd 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 3rd 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

Vinyl chloride-induced tumors have been found to have increased concentration of antigenic coagulation Factor VIII. Factor VIII has, previous to our studies, been demonstrated by Hoyer *et al.*, to be present in only three cell types, i.e., endothelial cells, platelets and megakaryocytes. These facts allowed the deduction as to which cell type becomes aberrant in hepatic angiosarcoma and allowed the identification of that cell type in frozen sections of diseased liver.

Examination of human liver from normal and angiosarcomatous individuals, using immunofluorescent microscopy and indirect fluorescent staining, was done. Sections from normal liver demonstrated a weak Factor VIII immunofluorescence in the sinusoidal linings with consistently stronger staining along linings of arteries and veins. In contrast, sections of the angiosarcomatous tissue showed intense fluorescence in the proliferating cells lining enlarged sinusoids. More importantly, strands of fluorescent cells appeared to be infiltrating the tissue.

Absorption studies indicated an increase in Factor VIII levels of angiosarcomatous tissue. Equivalent concentrations of anti-Factor VIII were mixed with two-fold serial dilutions of homogenized normal angiosarcomatous liver. Angiosarcomatous extract inhibited the antibody by up to 16 times greater dilution than normal extract, thus confirming the increased concentrations of Factor VIII in this tumor. These observations are evidence that angiosarcoma is an endothelial cell tumor rather than a Kupffer cell tumor and suggests that the aberrant cells may have an increased production or storage capacity for Factor VIII or a decreased transport from the cell.

A compilation of HLA frequencies in VC workers is continuing to determine if a possible increased association of certain "tissue types" can be determined in individuals with angiosarcoma or chemically-related disease. To date, approximately 519 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.

An evaluation of immunocompetence of humans chronically exposed to vinyl chloride was done using lymphocyte transformation and rosetting techniques. These data indicate that there is no residual immunological depression as a result of chronic exposure to increased levels of vinyl chloride.

Technical Proposal B -- Biochemical Enzymatic Systems for the Detection of Vinyl Chloride and Other Chemical Injury and Cancer Development in Industrial Workers. J. T. Du

Biochemical studies in the livers of rats exposed to vinyl chloride have just been done (600ppm for 9 months). Consistent with our previous high dose exposure studies, an elevation of the non-protein sulfhydryl content (GSH) and glutathione reductase activity in the treated group has been found. However, the glutathione transferase activity (using 1,2-epoxy-3-(p-nitrophenoxy) propane; trans-4-phenyl-3-buten-2-one and p-nitrobenzyl chloride as substrates) showed no statistical increase even though the mean value is higher. This suggests that with elevated glutathione, the rats probably can handle the low level of toxic metabolites without induction of the transferase enzyme. The glucose-6-phosphatase activity in these vinyl chloride exposed rats, unlike the results with high dose experiment, was not significantly lower than the control.

Technical Proposal C -- Glycosaminoglycan Changes as a Possible Aid in the Early Detection of Fibrotic Injury and Cancer of the Liver. C.E. Kupchella

A manuscript concluding that GAG profiles may reflect active liver disease is in final draft.

An article describing our assessment of the relationship between tumor glycosaminoglycan patterns and the behavior of tumors (using metastatic and non-metastatic variants of the Morris Hepatoma model) is in final typing.

In November we presented the histochemical portion of this study at the Annual Meeting of the Kentucky Academy of Science.

The biochemical/analytical portion of the study was done by Elaine Drake, a junior medical student. In October, she won first-place among medical students in the Research Day competition at the University of Louisville School of Medicine. Ms. Drake's paper entitled "Glycosaminoglycan Patterns in Six Behavioral Variants of Morris Hepatomas" has been submitted for presentation at the Annual meeting of the American Federation for Clinical Research in Washington, D.C. in May 1980.

Technical Proposal D -- Histological Systems of Detection. C.H. Tamburro, R. Schrodt, G. Barrows

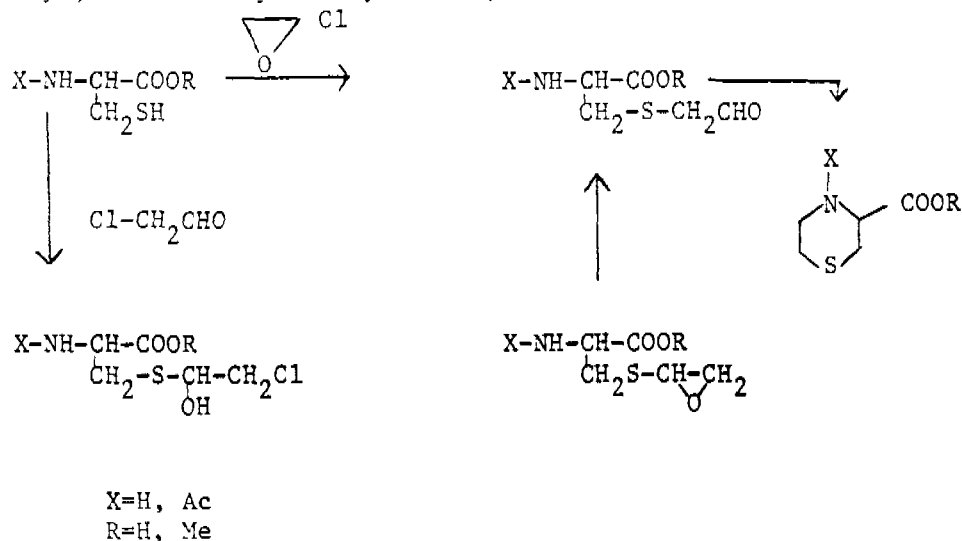
Fourteen normal autopsy specimens with total of 4 biopsies each (total 56 biopsies) have been accessioned with age ranging from 14 to 71 years. These patients have no evidence of significant clinical liver disease and therefore are providing good normal data.

Two computer programs have been developed for use with the Digitizer. The first program integrates the total area contained in designated segments of the biopsy. The second uses a sampling technique to calculate area. Preliminary appraisal suggests the second technique will be faster and more accurate. The evaluation of computer reproducibility compare favorable with manual techniques. Less than .01% intra-assay error was observed. While the determinations on normal patients are not yet complete, preliminary data reveal a mean of 1.25% collagen with a range of 0 to 6.1%. Increase appears to be dependent with age. The most predominant area of increase associated with age has been the midzonal areas of the liver.

The 110 biopsies from vinyl chloride exposed workers and their collagen data will be compared with that obtained from normal patients, particularly with relation to site of biopsy.

Technical Proposal E -- Chemical Systems of Detection of Toxicity of Vinyl Chloride. J. L. Wong

The comparative study of the reaction of chlorooxirane and chloroacetaldehyde with sulfhydryl compounds has now been completed. Their reaction pathways with cysteines (unmodified, N-acetyl, and N-acetyl-methyl ester) are delineated below.



Although both routes are converged to yield the cysteine S-acetaldehyde conjugate, their reaction rates are vastly different. In general, chlorooxirane completes its reaction within an hour while chloroacetaldehyde requires overnight. These chemical observations can be applied in (1) specific assays for the two putative metabolites in cellular studies of vinyl chloride, and (2) detoxification mechanism of cells exposed to the vinyl carcinogen.

The study of the putative action of vinyl chloride is continued in respect to the physicochemical properties of the etheno derivatives of cytidine, adenosine, and guanosine - products of the reaction between chloroacetaldehyde and nucleic acids. The ^{13}C -FTNMR spectroscopic properties are particularly revealing. By means of deuteration and proton undecoupling technique in acquiring the carbon-13 spectra, the various carbon chemical shifts are assigned and the ^{13}C - ^1H coupling constants determined. From the latter data, the conformational preferences of the sugar moiety in the modified nucleosides are deduced as follows:

(1) anti conformation for ethenocytidine and (2) syn for etheno-adenosine. Since anti conformation of nucleosides is necessary for the formation and stability of the helix, the influence of the etheno bridge on the adenine nucleus in altering the ribosyl group to syn will impose considerable stress on the DNA chain which might result in biological damages.

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

In the last quarter, our efforts have been two-fold. First, we are continuing studies on the interferon assay. We have obtained a list of chemicals from the DHEW, all of which will be tested to determine the validity and sensitivity of this assay when compared to available carcinogen assays. We have also further explored the molecular mechanism behind the assay. We have shown that our chemicals need to be microsomally activated in order to inhibit interferon induction, since addition of reduced glutathione with the carcinogen results in an abrogation of the effect of the carcinogen on interferon induction. Initial studies with leupeptin, an inhibitor of protease activity which can enter mammalian cells, indicate that destruction of proteases restores interferon production. Thus, protease may play a role in the carcinogen-interferon interaction.

Secondly, we are developing a bacteriophage (virus) model to study carcinogenic mechanisms. Briefly, we have purified DNA from a small bacterial virus (DNA mol. wt. 10^7) and will expose this DNA to carcinogens (specifically methylating agents). Then, sensitivity of modified DNA to DNA-specific enzymes, and the viability of this DNA in transformation assays will be determined. Our ultimate aim is to correlate specific DNA Lesions with a specific defect in DNA expression.

Several papers we have published bear on this type of study; i.e., developing systems whereby this study will be facilitated. All of these have acknowledged MCA support and will be included with the final report.

Technical Proposal G -- Tissue Antigens and Antibodies in the Detection of Vinyl Chloride Injury. Enrique Espinosa

During this quarter the human hepatoma cells (PLC/PRF/5) grown in culture were continued to be studied for antigen production using double immunodiffusion and indirect immunofluorescent procedures. New antigens tested included alpha fetoprotein, liver specific F-antigen, clotting Factor VIII, and serum immunoglobulin G. It was demonstrated that these liver malignant cells are not involved in the synthesis of these proteins.

In order to confirm the effective production of serum proteins albumin, fibrinogen, transferrin, alpha 1-antitrypsin and alpha 2-macroglobulin demonstrated in work done during the second quarter, control experiments using immunofluorescent absorption tests were devised. For these absorption tests purification of the human antigens under investigation was required. This was accomplished by chromatographic procedures. By employing the purified human protein fractions in the immunofluorescent absorption tests, we could confirm production of the serum proteins by hepatoma.

In order to extend the above observations to other individual human hepatomas, part of this quarter was also used in attempts to culture hepatoma cells obtained from 2 other patients. Cells from one of these patients have successfully grown in culture and we are currently studying their nature.

Technical Proposal H -- Disposition of ^{14}C -Vinyl Chloride in Mice by Whole-Body Autoradiography. William J. Waddell

Plans have been completed to study the distribution of ^{14}C -vinyl chloride in mice by whole-body autoradiography in a collaborative experiment with Dow Chemical Co. Dr. Phil Watanabe and his colleagues will convert 1 mCi of ^{14}C -dichloroethane, obtained from New England Nuclear, to ^{14}C -vinyl chloride in their laboratories at Dow in Midland, Michigan. Immediately after synthesis, the gas will be used to expose 5 male mice for 3 hours in a closed chamber. The mice will then be transferred to an atmospheric environment free of vinyl chloride and which continually removes any expired radioactivity. One of the mice will then be frozen, after brief anesthesia with ether, by immersion in dry ice/hexane at 0.33, 1, 3, 9, and 24 hours. The mice will be packed in dry ice and transported to the laboratory at the University of Louisville for processing by whole-body autoradiography for soluble compounds. This technique does not allow thawing or the use of any solvents; consequently there is no translocation or loss of radioactivity.

The date of the acute experiment is scheduled for April 1, 1980; the processing for autoradiography will take approximately 3-6 months.

The experiment is intended to reveal the sites of retention of the metabolites of vinyl chloride. Particular attention will be focused on the distribution of radioactivity within the liver, but all organs in the body will be examined for localization of radioactivity. All of the unchanged vinyl chloride will be expelled from the animal and only non-volatile metabolites or those that have reacted with cellular constituents will remain. It is hoped that the cell types which metabolize and/or retain the metabolites can be identified and therefore inferences on the mechanism of tumor formation can be made.

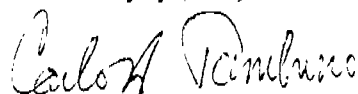
Technical Proposal I -- Vinyl Chloride Metabolism in Isolated Liver Cells,
Richard C. Feldhoff

The liver perfusion apparatus has been used collaboratively by Drs. Du and Feldhoff to prepare parenchymal and endothelial cell populations. Collagenase or pronase perfusion procedures were used. Techniques are being modified to optimize cell yields and viabilities. The time and concentration dependence of two suspected chemical inhibitors of albumin secretion were investigated with the results quantitated by rocket immunoelectrophoresis.

In another set of experiments, a procedure for the quantitative preparation of unaggregated polysomes by Mg(II) precipitation has been developed. The effects of ethanol and various chemicals on albumin synthesis and secretion and on intracellular polysome aggregation are being investigated.

This completes the 3rd 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,



Carlo H. Tamburro, M.D.
Professor of Medicine
Chief, Division of Digestive
Diseases and Nutrition