marker for vascular lining cells of the endothelial type. Further experiments in animals gave us results indicating that normal endothelial cells found lining the liver sinusoids had little, if any, Factor VIII fluorescence (content). Endothelial cells found lining larger vessels, on the other hand, demonstrated a striking fluorescence as did experimentally transplanted mouse angiosarcomas. Conversely, mouse hepatic Kupffer cells, a second type of hepatic lining cells, distinguished in histological cross section following engorgement with carbon particles, failed to demonstrate positive fluorescence. An increase in antigen Factor VIII in hepatic angiosarcomatous endothelial cells as compared to normal, leads us to believe that the aberrant cells have increased production or storage capacity for antigenic Factor VIII.

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

Staining techniques have been applied to various liver cell types to help identify the isolated liver cells. In the non-hepatocytes, the Kupffer cell is the peroxidase positive and the endothelial cell is the peroxidase negative cell. In a non-hepatocyte preparation, we found 18% peroxidase positive. After further separation of the non-hepatocytes by elutriation, only about 1% of the endothelial cell fraction was peroxidase positive.

We also did some experiments feeding chloroethanol, a vinyl chloride metabolite, to rats (30 mg/kg/day for 10, 20 and 40 days). The non-protein sulphydryl content, (mostly glutathione), was increased after 20 days, but the glutathione-related enzyme activities, glutathione transferase and glutathione reductase, were not altered, suggesting either the dose is not high enough or that chloroethanol and vinyl chloride metabolize via different routes. Further experiments with higher doses will be performed to elucidate the mechanism.

Technical Proposal C -- Glycosaminoglycan Changes in Earlier Detection of Fibrotic Injury and Hepatic Cancer. C.E. Kupchella

The original aim of this study was to determine the usefulness of urinary and tissue glycosaminoglycan (GAG) measurements in the detection of chemically-induced liver injury. We have made substantial progress toward this end and this has been described in previous reports. Within the period July 1979 - September 1979, specifically, we accomplished the following:

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

The means of analyzing light electron microscopic sections of liver tissue quantitatively for the amount of collagen (scar tissue) in individuals exposed to vinyl chloride has demonstrated a great variation in the amount of stainable collagen present. This can vary from non-detectable collagen without the use of special collagen stains, such as the trichrome, to 35-40% of the total biopsy being occupied by fiberous tissue in the terminal stages of the disease. At this point all 110 biopsies from the vinyl chloride workers have been reviewed as to the degree of collagen deposition present. Morphometric analysis previously had been delayed in order to develop standards for the normal human collagen content at various ages. The study of the normal distribution of collagen content at the various ages in individuals without history of chemical, viral or medical disease, or injury to the liver, has had 14 individuals accessed. Approximately 4 are in the under 25 age group, 6-7 in the 25-40 age group, and 3 in the 40-50 age group. Preliminary data demonstrates an increase in the collagen deposition in the normal human liver associated with age. In the younger age group less than 1% of the studied area is occupied by stainable collagen. In the older age group stainable collagen in the sinusoidal areas may range as high as 5-6%. There is considerable overlap between individuals but close agreement between biopsies in the same individual. At present, the lower age ranges has sufficient cases to develop standards. A few additional cases are needed in the upper ranges. The acquisition of these additional cases depends on the availability of accidental deaths occurring in normal individuals of the older age group without evidences of hepatic disease. However, sufficient data is available for us to resume the morphometric analysis of the vinyl chloride exposed worker's liver biopsies utilizing the Hewlett-Packard 9864-A digitizer and a 9815-A micro computer.

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

In the previous report we have delineated the reaction pathways of chlorooxirane with 3,4-dichlorobenzenethiol and N-acetylcysteine. In both cases, the major product is the sulfur conjugate S-acetaldehyde. Since chlorooxirane can spontaneously rearrange to chloroacetaldehyde, we have proceeded to investigate the reaction intermediates of the latter in the detoxification by cellular sulfhydryl compounds. The reaction of chloroacetaldehyde with N-acetylcysteine under controlled pH conditions in aqueous medium at 0°C produced an intermediate compound. Upon warming up the reaction mixture to room temperature, our previously identified thiazene was isolated as the final product. In order to

1) Benzo-(a)-pyrene, #4 fraction of tobacco smoke condensate, 1,2-dimethylbenz (a) anthracene, 2-aminofluorine, aflatoxin-B_1, and styrene oxide all inhibited the induction of interferon by Newcastle disease virus.

2) MMS, a highly carcinogenic mutagen, inhibited the induction of interferon, while its analog, EMS, a rarely carcinogenic mutagen, had no effect on interferon induction.

3) Chloroacetaldehyde, the metabolite of vinyl chloride that is believed to be responsible for the actual carcinogenic event perpetrated by vinyl chloride, inhibited the induction of interferon by virus, but chloroethanol and chloroacetic acid, benign metabolites of vinyl chloride, had no effect on interferon induction.

These results suggest that the inhibition of interferon induction by chemicals may be a useful marker of the carcinogenic potential of a chemical, after extensive further study and collaboration of the results. The results were presented at the 1979 Annual Meeting of the American Society for Microbiology and at the International Symposium on Interferon of the Wadley Institutes of Molecular Medicine, and will appear in the form of two manuscripts. The Manufacturing Chemists Association has been recognized for its support to the completion of the data in the manuscripts.

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

In order to further investigate antigen production by hepatoma application of the indirect immunofluorescent procedure to this question was investigated during this quarter. To accomplish this, several conditions for optimal growth of tumor cells sheets (PLC/PRF/5) in culture were studied. Satisfactory results were obtained by growing stationary cultures on coverslips in Leighton tubes at 37°C in a modified Eagle's medium containing 10% fetal calf serum. Cell morphology appeared excellent after 1/2 to 1/3 of confluency had developed in the coverslips. This was accomplished following seeding of 300,000 cells and culturing for 5-6 days. For the immunofluorescent staining the coverslips were then rinsed in Eagle's medium at room temperature and in cold ethanol in order to get rid of medium proteins. After fixation in ethanol (-75°C) for 10 minutes, The cells were tested for presence of several serum proteins (albumin, fibrinogen, transferrin, alpha 1-antitrypsin and alpha 2-macroglobulin). Specificity of the staining was