

TECHNICAL PROPOSAL B

**BIOCHEMICAL ENZYMATIC SYSTEMS FOR DETECTION
OF VINYL CHLORIDE AND OTHER CHEMICAL INJURY AS
A POTENTIAL MEANS OF DETECTION OF CANCER IN HUMANS**

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Characterization of the biochemical and enzymatic changes occurring in progressive exposure in animal experiments will be continued. This includes the following studies:

- (1) The determination of microsomal P-450 content, the cytochrome protein that is responsible for the oxidation of vinyl chloride;
- (2) The activity of mixed function oxidase; i.e., the capacity for the microsomes to metabolize drugs (N-demethylation);
- (3) The activity of glucose-6-Pase, a gluconeogenic enzyme and a marker for preneoplastic change;
- (4) The activity of glucose-6-P dehydrogenase, a pentose shunt enzyme (an increase of this can lead to more ribose and nucleic acid biosynthesis;
- (5) The level of reduced glutathione in liver for detoxification of carcinogenic metabolites; and
- (6) The activity of glutathione reductase. This enzyme activity indicates the capacity to regenerate and maintain the level of reduced glutathione. An increase can be an early alarm of the animal for overexposure to the toxic agent, indicating possible early hepatocellular injury.

These studies will enable us to identify the sequential cellular enzymatic changes occurring from early injury to tumor development. As has already been reported, the changes in gluconeogenesis and in the pentosephosphate shunt already parallel the alteration seen in primary hepatocellular tumors. The present working hypothesis for the non-development of primary cell tumors in the adult animal is the ability of the adult hepatocyte to adequately detoxify the carcinogenic metabolites of vinyl chloride, chlorooxirane and chloroacetaldehyde. The adjacent cells, sinusoidal lining cells, as well as newborn liver cells, most likely have a decreased ability of detoxification. In this or similar manner, these cells have a greater susceptibility to DNA injury

due to the prolonged exposure of chloroacetaldehyde to the DNA molecule. This would explain why angiosarcomas occur in adult rats and primary hepatomas occur in newborn rats, as has been recently shown by Maltoni.

In our next stage of animal exposure experiments, we will add weanling rats to the vinyl chloride exposure animal group. In these animals, enzymatic assays to document this hypothesis will be performed in a manner similar to the adult rats. Both weanling and adult rats will be utilized for verification and further elucidation of the immunological and tissue antigen test systems by Espinosa's group. Urines from these same rats will be utilized to further verify the urinary glycosaminoglycan studies by Kupchello's group and the biological end-product analysis by Wong and Taylor.

In addition, we plan to investigate two more enzymes related to glutathione and detoxification:

- (A) Gamma-glutamyl transpeptidase (GGTP) - This enzyme is responsible for the breakdown of glutathione. It has also been used as a circulating enzyme for the detection of early hepatocellular injury. It has, under recent years of clinical evaluation, lost some of its clinical importance due to the high frequency of an elevation of its activity in circulating blood in the absence of any documentable hepatocellular injury. The activity of this enzyme in the liver has been found to increase considerably during several cases of chemical carcinogenesis. Higher levels of the enzyme have also been seen in patients with either primary or secondary neoplasms. The possibility that the high enzyme activity in blood might reflect more breakdown of glutathione rather than hepatocellular injury is under evaluation. It may well be that the "falsely positive" elevated GGTP levels in vinyl chloride-exposed individuals without evidence of hepatocellular injury may really reflect a disturbance in the glutathione levels and indicate an impairment in the detoxification of the intermediate metabolite, chloroacetaldehyde, which subsequently leads to hepatocellular injury.
- (B) Aralkyl glutathione-S-transferase - The activity of this enzyme can be used to indicate the capacity for detoxification of vinyl chloride metabolites in the rat. The possibility that this might be a better predictor of whether the animal is more or less or equally susceptible to vinyl chloride injury at various exposure levels is the goal of this investigation. These studies in parallel with circulating glutathione reductase will give us a better understanding of the detoxifying capability of the

animal and, hopefully, we may be able to extrapolate the findings to man to predict better about the worker's detoxifying capacity. There has been some thought that manipulation of the diet may add to the increased ability to form these enzymes for better capacity of detoxification. This potentiality is, at present, most speculative; and, if the previously stated investigations warrant, it would be followed by subsequent studies.

The final portion of this study will include the electron and light microscopic studies of these animals exposed to determine the graded morphological changes due to vinyl chloride exposure and to compare these to the enzymatic results already determined. These tissues have already been prepared for analysis, and the first set is now being studied. These include selected groups from 28, 71, and 103 hours of exposure with their paired controls. Similar studies will be performed with the next group of more prolonged exposures. We feel strongly from our human studies that these animal electron microscopic evaluations will greatly assist us in understanding the changes we see in our human studies being performed by Drs. Schrodtt and Tamburro.