DRAFT MEMO FOR CANCER APPLIC.

To: Professor J. Lederberg

From: Alan Duffield

Subject: Applications of Gas Chromatography-Mass Spectrometry to Identify Biochemical Markers which may presage the Development or Indicate the Presence of Cancer.

Using the existing instrumentation and experience of this laboratory in the analysis of body fluids by gas chromatography mass spectrometry I would suggest two approaches for research on the above topic.

I. Use of Gas Chromatography-Mass Spectrometry for the Identification of New Metabolic Idiosyncrasies of Cancer.

This approach would use the gas chromatogrph-mass spectrometer combination for the analysis of a statistically significant number of body fluids from clinically diagnosed cancer patients and normal controls. From this study a correlation of different metabolites between the two groups could be determined. Each body fluid could be assayed for extractable organic acids, amino acids and carbohydrates as we are presently doing for children with suspected diseases of a genetic origin. The analysis time would be about one hour per fraction or three hours per body fluid. Data retrieval from the computer system and interpretation would limit sample throughput to about two per eight hour day.

This methodology will assay for any compound which after derivatization

will elute from a gas chromatographic column. The interpretation of the generated mass spectra will be enhanced by the library search routines currently under development, in this laboratory. As you know the literature is contradictory on which metabolites may constitute viable markers for the detection of cancer. Thus a voluminous literature exists for the association of various tryptophan metabolites (3-hydroxy-kynurenine, xanthurenic acid, 3-hydroxy-anthranilic acid) with the presence of cancer and other research disputes such a relationship. A similar dichotomy exists in the literature for the excretion of β-aminoisobutyric acid from cancer patients. Some of these discrpancies might be expected to arise from the techniques used for the identification and quantitation of these metabolites. Indeed it was this last thought that stimulated our development of a new technique for the quantitation of β-aminoisobutyric acid in the urine of children with leukemia (W. E. Pereira, R. E. Summons, W. E. Reynolds, T. C. Rindfleisch and A. M. Duffield, Clin. Chim. Acta, in press).

It would appear that for every metabolite touted as an indicator of cancer there appears evidence to the contrary. This situation probably reflects the absence of suitably specific methods of metabolite identification in the past. At the present time gas chromatography-mass spectrometry must be the most powerful technique available for the identification of the metabolites present in body fluids.

II. The Use of Quadrupole Mass Fragmentogrphy for the Quantitation of known Metabolites whose concentration could be associated with Cancer.

It is possible that cancer might modify body biochemistry to the extent that some of the usual metabolites are present in body fluids in concentrations different from that present in the normal population. The question then is how to quantitate for specific metabolites present in a complex suite of metabolites isolated from body fluids. One answer to this problem is our recently developed technique of quadrupole mass fragmentography. I suggest the following as topics worthy of investigation by this technique.

Amino Acids. Several investigators have raised the point that some amino acids (methionine, histidine) occur in the urine in above normal concentrations in cancer patients. I propose to assay cancer patients and normals in order to define as precisely as possible the urinary excretion levels for both groups. From this correlations between amino acid concentration and cancer might appear.

Polyamines: Increased levels of spermidine, spermine and putrescine have been associated with cancer. As the techniques used for the quantitation of these compounds in urine has been non-specific (TLC, gas chromatography) I propose to develop a new quantitative assay involving the use of quadrupole mass fragmentography and deuterated analogs of these bases as internal standards. I am confident that this technique will allow quantitation below the nanogram level for these three bases and would thus be an advance on the methods currently used.