

RFP TITLE:

BIOCHEMICAL MARKERS THAT MAY PRESAGE
THE PRESENCE OF CANCER

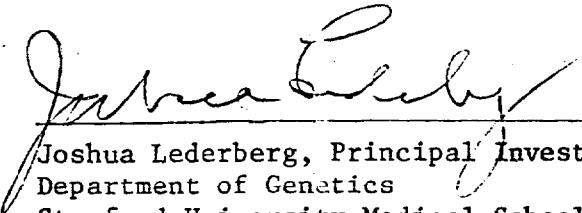
RFP NO.

NCI-CB-74-29 (PROJECT NO. CB-43902-S)

AMENDED PROPOSAL (JUNE 18, 1974)

SUBMITTED BY

THE BOARD OF TRUSTEES OF THE LELAND STANFORD, JR. UNIVERSITY



Joshua Lederberg, Principal Investigator
Department of Genetics
Stanford University Medical School

INTRODUCTION

The aim of this proposal is to use gas chromatography (GC) in conjunction with computerized mass spectrometry (MS), to investigate the presence of metabolites and metabolic changes occurring in biological fluid that may presage the presence of cancer.

In writing up this amended proposal we have taken into consideration the valuable comments and suggestions put forth by the site visit committee. Their suggestion accords with our own criticisms to narrow the emphasis to the screening of small selected groups of patients with specific types of cancer. In addition we briefly describe these segments of our research protocols which we modified after discussion with the site visit committee.

We propose using our existing analytical procedures, and also by developing new methodology, to begin studying limited populations of patients with the following types of cancer:

- (a) prostatic cancer
- (b) bladder cancer (multiple, recurrent, stage A carcinoma)
- (c) diagnosed leukemia
- (c) non-Hodgkin's lymphomas

In addition we are pursuing, at the suggestion of the site visit committee, ways of obtaining urine specimens from a relatively small number of adult patients with Hodgkin's Disease and others with cancer of the pancreas. As these samples materialize they will be screened for their metabolic content.

Specifically we intend to screen 10-12 urine samples from patients with each of these cancers and for a suitable number of controls (see below) for biochemical markers indicative of the disease state. In addition we will demonstrate the methodology required for the sub-nanogram quantitation of urine polyamine levels in order that this technique will be available for exploitation in subsequent years of this contract. We also intend to monitor the urinary beta-aminoisobutyric acid levels in children with diagnosed leukemias and non-Hodgkin's lymphomas.

AVAILABILITY OF BIOLOGICAL SAMPLES

Biological specimens will be made available to us by the Clinical Research Center and the Urology Clinic of the Stanford University Medical School through cooperation of Dr. W. Fair (Associate Professor of Surgery), and Dr. T. Long (Children's Hospital, Oncology Division, Stanford University).

METHODOLOGY

The gas chromatograph-mass spectrometer (GC-MS) system will be primarily used to separate and identify biological metabolites present in the urine of cancer patients. It is reasonable to assume that some metabolites that presage the presence of cancer may be present in extremely minute amounts (picogram levels). For the purpose of their detection and quantitation we propose using the technique of mass fragmentography (see original proposal). In the examples of urinary polyamine levels, where the sensitivity of detection is crucial, we intend to develop new analytic techniques capable of quantitating these compounds below the nanogram level.

SPECIFIC AIMS AND OBJECTIVES

An ancillary objective, in addition to looking at metabolic profiles of cancer patients (introduction a,b,c,d), will be directed towards a quantitative investigation of the following two biochemical markers that have been reported to be present at elevated concentrations in various types of neoplastic disease. This will involve accurate quantitation of the following urinary metabolites:

- 1) Polyamines in prostatic cancer
- 2) Beta aminoisobutyric acid (BAIB) in leukemia (see original proposal).

The development of more sensitive analytic methods is aimed at answering questions of the mechanisms of the increases in polyamines noted by Dr. Fair. This would entail comparisons of prostatic fluid, voided urine and bladder urine from his patients. If tissue samples of 100 mgs or less can be assayed, surgical specimens can be examined--larger samples interfere with necessary histopathological scrutiny.

SCREENING URINE SAMPLES FOR BIOCHEMICAL MARKERS

Urine from patients afflicted with the four types of cancer listed in the introduction will be screened by GC/MS procedures as detailed in our formal proposal.

Bladder cancer, specifically multiple, recurrent, stage A carcinoma, presents a special opportunity for metabolic analysis. The multiple recurrent lesions suggest a systemic basis for the disease; hence a theoretical rationale for metabolic screening and perhaps genetic etiologies. The Urology Department sees almost 50 cases per year of this condition and we foresee no difficulty in collecting about 12 for a one-year protocol. The sample collections and protocol regimens will correspond to those in the prostatic cancer series, who will also serve as adjunct controls. Similar comments apply to "normal" controls. (One must keep in mind that most normal middle aged men are subject to some stage of neoplastic disease of the prostate).

For this condition we will place special emphasis on family studies. In a preliminary search for signature substances, we will scan siblings, spouse and off-spring of the propositus where available. If such substances are identified they will be systematically tracked through pedigrees by conventional genetic methods.

CLINICAL PROTOCOL FOR SCREENING URINE SAMPLES FOR BIOCHEMICAL MARKERS

(a) Collection of samples. All urine samples will be 24 hour collections. The urine will be collected in sterilized glass bottles under toluene and frozen immediately until ready for use.

(b) Prestudy regimen. Urine specimens will be obtained from patients who have discontinued all medication for at least 48 hours prior to collection. Dietary controls would not be possible on non-hospitalized patients. The patients selected will be free of fevers, emaciation, anemia, uremia or malaise. Where possible all patients will be surgically staged. In other instances clinical records will be used to provide data on the extent of the disease. The study groups for each of the four categories of disease will consist of between 10 and 12 patients.

(c) Controls. These will be matched according to age, weight, sex and smoking habits.

(d) Intervals of Study. 24-hour urine collections will be repeated twice at suitable intervals (30, 60 day) following the initial collection.

1) Polyamines in Prostatic Cancer

Carcinoma of the prostate ranks second among the causes of male cancer deaths in the United States (1). At present there is no accurate method for early detection of prostatic cancer. It has been found that the polyamines putrescine [$H_2N \cdot (CH_2)_4 \cdot NH_2$], spermidine [$NH_2 \cdot (CH_2)_3 \cdot NH \cdot (CH_2)_4 \cdot NH_2$] and spermine [$NH_2 \cdot (CH_2)_3 \cdot NH \cdot (CH_2)_4 \cdot NH \cdot (CH_2)_3 \cdot NH_2$] have a strong affinity for the nucleic acids RNA and DNA (2). Increased levels of polyamines

have been reported in rapidly growing neoplastic tissue (3) and in the urine of patients with different types of cancer (4). Since the highest concentrations of the polyamines found in the human body occur in the prostate (5), it would be significant to find if a correlation exists between carcinoma of the prostate and urinary excretion levels of the polyamines. This study would provide information concerning the urinary concentration of polyamines which might then be related to the presence of carcinoma of the prostate.

Initial studies by Dr. W. Fair's group in the Department of Urology, Stanford University Medical School, have shown (using paper electrophoresis) that urinary levels of spermidine appear to be significantly elevated in many patients with prostatic neoplasms. Following our discussion with the site visit committee we propose to expand on the initial investigation of Dr. Fair by developing a methodology for the sub-nanogram quantitation of the polyamines, putrescine, spermidine and spermine. If this methodology can be developed and tuned for optimum sensitivity we will be in a position to resolve the conflicting evidence present in the literature on the efficacy of urinary polyamine levels as markers for the detection of cancer.

Preliminary studies we have made on the synthetic TFA-polyamines have shown that the chemical ionization mass spectra of these compounds are ideally suited for mass fragmentography. The electron impact spectra of these compounds do not show molecular ions, but show extensive fragmentation resulting in generally low abundance ions, which do not result in optimum sensitivity using mass fragmentography. On the other hand, these polyamines are good proton acceptors and their TFA derivatives give abundant quasi-molecular ions ($M + 1$), in the chemical ionization mode making them an ideal derivative for quantitation by mass fragmentography. Since the polyamine levels in urine are extremely low ($< 1-2$ mg/24 hrs.) we feel that chemical ionization-mass fragmentography is the most promising method available for the quantitation of these biochemical markers. To this end we have approached instrument manufacturers for time to use their instruments to verify the feasibility of our intended assay as we do not have access to a chemical ionization mass spectrometer. In the first year we would demonstrate the feasibility of a chemical ionization-mass fragmentography methodology for sub-nanogram quantitation of putrescine, spermidine and spermine. We would then begin to apply the technology to the routine determination of urinary polyamine levels in patients with prostate cancer and other carcinomas and for assaying polyamine levels in tissue obtained from surgical procedures.

CLINICAL PROTOCOL FOR INVESTIGATION OF POLYAMINES IN PROSTATIC CARCINOMA

(a) Collection of samples. All urine samples will be 24-hour collections. The urine will be collected in sterilized glass bottles under toluene, and frozen immediately until ready for assay.

(b) Prestudy regimen. Urine specimens will be obtained from patients who have discontinued all medication for at least 48 hours prior to collection. Dietary controls would not be possible on non-hospitalized patients. The patients selected will be free from fevers, emaciation, anemia, uremia or malaise. All patients chosen for the study will be surgically staged, so that the extent of the disease is accurately determined. The study group will consist of 10-12 patients with diagnosed prostatic carcinoma.

(c) Controls. Controls obtained from out-patient clinic volunteers will be matched according to age, weight, and smoking habits. In addition samples from other urology patients will serve as adjunct controls.

(d) Intervals of study. A 24-hour urine collection will be made on each patient prior to surgical staging and two more specimens collected after the patient undergoes surgery. The patient will be followed up in the Urology Clinic and 24-hour urine specimens obtained at appropriate intervals.

2) Beta Aminoisobutyric Acid (BAIB) in Leukemia and Non-Hodgkin's Lymphomas

During a preliminary screening program of the urinary metabolites of children with diagnosed leukemias, we observed several cases in which the levels of BAIB were substantially elevated ($> 1\text{g}/24\text{ hrs}$). Since existing procedures for the urinary analysis of BAIB were not specific enough, we developed a sensitive method for quantitation of BAIB in urine by Mass Fragmentography (6). BAIB has been shown to be one of the end products of DNA catabolism, and thymine is its principal precursor (7). In conditions such as neoplastic diseases, one might expect a relationship to exist between the excretion levels of BAIB and the DNA metabolic process.

We propose measuring BAIB levels, as an indicator of disease activity in the urine of children with newly diagnosed leukemias and non-Hodgkin's lymphomas. Details of the experimental protocols will be found in our original contract proposal.

CLINICAL PROTOCOL FOR INVESTIGATION OF BAIB IN LEUKEMIA AND NON-HODGKIN'S LYMPHOMAS

(a) Collection of samples. All urine specimens will be 24-hour collections. The urine will be collected in sterilized glass bottles under toluene, and frozen immediately until ready for assay.

(b) Pre-study regimen. Urine specimens will be obtained from patients who have discontinued all medication for at least 48 hours prior to collection. A record of the patients diet will also be obtained from the dietician at Children's Hospital, Stanford University. The patients selected for study will vary from two to fifteen years of age. Care will be exercised to select patients who do not have fevers,

emaciation, anemia, uremia or malaise. Groups of 10 to 12 patients with newly diagnosed leukemia, and 10 to 12 patients with non-Hodgkin's lymphomas will be used for the study.

(c) Controls. Controls for the two groups to be studied will be of similar age, sex and weight and will be chosen from patients in the Children's Hospital confined for treatment of other than disease. Urine specimens from the control patients will be provided by the Chief Resident of the hospital at Stanford University Medical School.

(d) Intervals of study. All urine specimens of patients in the two study groups, will be collected at intervals of 30 and 60 days following the initial collection.

ANCILLARY STUDIES ON TISSUE SAMPLES AND CELL CULTURES

There are two principal rationales for seeking biochemical signatures of cancer;

- 1) a constitutional -- genetic or environmental -- systemic predisposition, and
- 2) a direct metabolic effect of the incipient tumor mass itself.

In the foregoing presentation, 1) is represented by the planned studies on bladder cancer; 2) on prostatic.

Since the metabolic output of an incipient tumor, unless amplified by systemic control mechanisms, will be diluted in the whole body space, it may be difficult to identify in the overall urinary output or in plasma solution.

We therefore plan to include some pilot studies along the following lines:

- a) displacement of albumin-bound metabolites, as has been noted as a side-effect of uricosuric drugs, and
- b) scanning tissue specimens (surgical, biopsy, and cell cultures) for signature substances that may be expected to be found at higher concentrations in situ than in the body fluid. (Once such substances are identified, they can be sought more assiduously in the body fluid samples.)

In this connection, Professor Hilary Koprowski of the Wistar Institute, University of Pennsylvania, has offered to provide hybrid cell lines which are segregating for a single human chromosome that influences the neoplastic-transformation characteristic.

These studies are not expected to take a significant part of our effort under the present contract; but if successful they may be the basis of requests for support for larger scale efforts.

It should be noted that the epidemiological and experimental designs presented hereinabove are tentative predictions. Rigorous, detailed prospective efforts will be needed to substantiate the validity of any preliminary findings that may emerge from the presently contemplated effort; and these can be designed only on the basis of the information we are now seeking.

REFERENCES

1. American Cancer Society (1970). Cancer Facts and Figures; Biostatistics of the United States.
2. Tabor, H. and Tabor, C. W. "Spermidine, Spermine and Related Amines." Pharmacol. Rev. 16: 245 (1964).
3. Russell, D. H., Levy, C. C., Schimpff, S. C., and Hawk, I. A., "Urinary Polyamines in Cancer Patients." Cancer Res. 31, 1555 (1971).
4. Denton, M. D., Glazer, H. S., Walle, T., Zellner, D. C., and Smith, F. G. In Polyamines: Their Implications for Effective Cancer Regulation. National Cancer Institute Monograph, Raven Press, New York.
5. Mann, T.: The Biochemistry of Semen and of the Male Reproductive Tract, pp. 193-200. J. Wiley and Sons, Inc., New York, 1964.
6. Pereira, W. E., Summons, R. E., Reynolds, W. E., Rindfleisch, T. C., and Duffield, A. M., "The Quantitation of Beta Amino-isobutyric Acid in Urine by Mass Fragmentography." Clin. Chim. Acta 49, 401 (1973).
7. Fink, K., Henderson, R. B., and Fink, R. M., Proc. Soc. Exp. Biol. Med. 78, 135 (1951).
8. Young M. and Russell, W. T. "An Investigation into the Statistics of Cancer in Trades and Professions." Spec. Rep. Soc. Med. Res. Com. No. 99. London, 1926.
9. King, H., Bailar, J. C.: "Epidemiology of Urinary Blader Cancer." J. Chron. Dis. 19, 735 (1966).