BIOCHEMICAL MARKERS OR ENZYME CHANGES THAT MAY PRESAGE THE PRESENCE OF CANCER

CONTRACT NUMBER NO1-CB-43902

Progress Report for the period July 1, 1975 to January 31, 1976.

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This report discusses progress made during the above period and continuing to date on the two areas of emphasis of our recent research:

A. Development and application of an analytical method for quantitation of urinary polyamine levels.

B. Screening of urine for metabolites which might presage the presence of cancer.

A. Development of an Analytical Method for the Quantitation of Urinary Polyamine Levels.

A sensitive and specific method using mass fragmentography for the analysis of the polyamines putrescine, cadaverine, spermidine and spermine has been developed, along with a method of synthesis for their deuterated analogs. The procedure involves addition of a known amount of a standard solution of deuterated analogs to the urine, followed by overnight acid hydrolysis, butanol extraction and ion exchange chromatography on a strongly cationic ion exchange resin. These procedures have been described in detail in previous reports. The polyamine extract is triflouroacetylated, and polyamine quantitation is achieved by measuring peak height ratios of specific ions characteristic of the triflouroacetyl derivatives of the indigenous and deuterated polyamines, m/e 126 and m/e 154 being characteristic of the derivative of the indigenous material, and 128 and 156 being characteristic of the derivative of the deuterated analog.

After injection of the samples into the GC/MS system, approximately one-half hour is required for analysis, followed by five minutes for processing the analytical data. The concentrations of indigenous polyamines are expressed in mg/100 ml at the end of the time required for processing. During the actual GC/MS runs, the computer monitors the specific ions (126, 128, 154 and 156) characteristic of the materials being analyzed, and at termination of the runs, the total ion current (Fig. I) is printed out at a CalComp plotter.

During processing of the analytical data, the contributions of the individual specific ions to the total ion current are separated and displayed on a T.V.Monitor along with background subtraction (Fig. II), and the finding of the peak's maximum (Fig. III).

Before the actual urine analysis, several quantitative mixtures of pure deuterated polyamines and pure non-deuterated polyamines are made up and derivatized. These mixtures are analyzed by GC/MS and processed to determine the specific ion ratios for each polyamine. These ratios are then plotted versus relative concentrations of the nondeuterated and deuterated polyamines to establish calibration curves for the individual polyamines (Fig. IV). During the actual urine analysis, the calibration curves are used to determine the relative concentration of the non-deuterated indigenous materials, from the specific ion ratios determined. The concentrations of the polyamines in the urine can then be determined.

Our intent in pursuing this study is to determine if the polyamines can be used as markers for the early detection of prostatic cancer. Prostatic cancer was chosen, because the highest concentrations of polyamines in the human body can be found in the male prostate gland. After the method had been developed, it was applied to clinical analysis. To date, sixteen prostatic cancer urines, eight benign prostatic hypertrophy urines and nine control urines have been run. One Hodgkins urine and one breast cancer urine have also been run. Three BPH urines, one prostatic cancer urine and three control urines have yet to be run. We have yet to process the analytical data. The clinical data on all of these patients, including controls, is included in this report. More extensive clinical data is available for inspection.

TABLE I. Summary of Data Available for Polyamine Analysis

Lab. No.	Disease	Age	Urine Volume	Chemistry	GC/MS	Final Anal.
229	Ca. Prostate	-	1100 cc	x	x	
238	Normal	51	1410 cc (12 hr)			
239	Normal	70	1840 (12 hr)			
302	Ca. Prostate	58	1610 cc	x	x	
324	Hodgkins	29	632 cc (12 hr)	x	x	
329	Ca. Prostate Grade II	69	1135 cc	x	x	
330	Ca. Prostate Grade I	61	2370 cc	x	x	
336	BPH	56	1300 cc (12 hr)	x	x	
337	BPH	61	1790 cc	x	x	
340	BPH	64	1430 cc (12 hr)	x	x	
343	BPH	-	-	x	x	
344	BPH	62	990 cc (8 hr)	x	x	
345	BPH	62	745 cc	x	x	
347	BPH	71	1250 cc (16 hr)	x	x	
348	BPH	71	372 cc (8 hr)	x	x	
349	BPH	51	900 cc (8 hr)	x	x	
351	BPH	51	590 cc (8 hr)	x	x	
354	Ca Prostate Grade II	51	3220 cc	x	x	
356c	BPH	52	620 cc (8 hr)	x		
362	Ca. Prostate Grade II	64	1425 cc			
363	Ca. Prostate Grade II	74	1550 cc	x	x	
1-74	Ca. Prostate Grade II	63	970 cc (12 hr)	x	x	
2-74	Ca. Prostate Grade III	64	720 cc (12 hr)	x	x	
3-74	Ca. Prostate Grade III	65	560 cc (12 hr)	x	x	
4-75	Ca. Prostate Grade III	56	440 cc (12 hr)	x	x	
5-75	Ca. Prostate Grade I	65	525 cc (12 hr)	x	x	
6-75	Ca. Prostate Grade III	54	620 cc (16 hr)	x	x	
7-75	Ca. Prostate	66	407 cc (12 hr)	x	x	
8-75	Breast Ca.	50	1240 cc	x	x	
9-75	Ca. Prostate	50	695 cc (12 hr)	x	x	
10-75	Ca. Prostate	51	2140 cc	x	x	
11-75	Ca. Prostate	61	475 cc (12 hr)	x	x	
12-75	Ca. Prostate	?	1050 cc (12 hr)			
1	Control	39	837 cc	x	x	
2	Control	33	1760 cc	x	x	
3	Control	39	1460 cc	x	x	
4	Control	35	1758 cc	x		
5	Control	27	930 cc	x	x	
6	Control	26	1020 cc	x	x	
7	Control	41	1248 cc	x	x	
8	Control	43	2030 cc	x	x	
9	Control	35	1430 cc	x	x	
10	Control	?	1330 cc	x	x	

B. Screening of Urine for Metabolites Which Might Presage the Presence of Cancer

During the period covered by this report, we have essentially completed the GC/MS profiles on the six fractions of the organic constituents of urine from patients with a variety of cancers. Subsequent preliminary computer analysis of the data is also essentially complete. The status of each of the samples provided to us by Dr. Waalkes of the National Cancer Institute is summarized in Table II. We are currently trying to obtain from Dr. Waalkes, now at Johns Hopkins, the patient histories corresponding to these samples.

As before, the urine from each of these patients was fractionated into: (1) an acidic and neutral fraction; (2) an amino acid fraction and (3) a sugar fraction. The acidic and neutral fraction was divided into two equal portions, one of which was methylated with diazomethane (D-OME) while the other was silylated with BSTFA + 1% TMCS (D-TMS). The sugar fraction was derivatized to the TMS derivative (S-TMS) with TRI-SIL-Z.
The amino acid fraction was also divided into two equal portions with one portion silylated with BSTFA + 1% TMCS (E-TMS) and the other converted to N-TFA-O-n-butyl derivative (E-TAB). Details of the procedure have been presented in previous reports.

Each of the six fractions of each urine was then analyzed by the GC/MS/Computer system. Each fraction yields about 600 complete mass spectra These spectra are processed by a computer program, called "CLEANUP", which is designed to detect components and remove from the spectrum of each component interference from background, column bleed and overlapping components. This procedure yields spectra which are much more characteristic of the spectra of pure compounds than are the raw data. Each fraction may yield from 30-60 component mass spectra, a considerable data reduction from the original 600 spectra, many of which are background.

We have assembled libraries of mass spectra of known compounds by dividing an available collection of over 3000 spectra of compounds of biological interest into subclasses corresponding to the chemical fractions isolated in the above procedure. The appropriate library is searched for the spectrum of each component detected by CLEANUP. Spectra of components which were not matched to the library are examined further in collaboration with the NIH supported DENDRAL project for computer-assisted structure elucidation.

Preliminary manual examination of the above data has revealed large amounts of β-aminoisobutyric acid (BAIB) excreted in the urines of three of the six patients with lung cancer. We have previously reported the association of increased urinary BAIB excretion with several lukemic, bladder, prostatic and lymphoma forms of cancer. Although precise quantitation is not yet available (see below) for the other samples (Table II) which do not show such large amounts of BAIB, the frequency with which this material appears in grossly elevated amounts in the samples we have examined is remarkable.

Table II. Status of Analysis of Organic Constituents of Urines of Patients with Various Cancers.

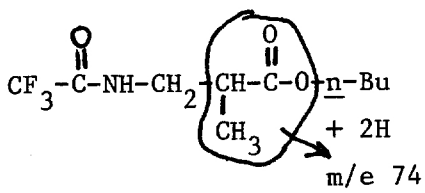
Breast Cancer	Chemistry	GC/MS Analysis	Computer Analysis
154 (007)	Completed	Completed	Completed
383(152)	"	"	"
282(084)	"	"	"
953(432)	"	"	"
751(343)	"	"	"
448(193)	"	"	"
Lung Cancer			
306	Completed	Completed	Completed
482	"	"	"
511	"	"	"
586	"	"	"
639	"	"	"
779	"	"	"
Pancreas Cancer			
314(110)	Completed	Completed	Completed
387(156)	"	"	"
532(244)	except D-TMS	except D-TMS	except D-TMS
668(314)	except D-TMS	except D-TMS	Except D-TMS
1120(508)	Completed	Completed	Except E-TMS
752(349)	Completed	Completed	except E-TMS
Colon Cancer			
533(245)	Completed	Completed	except E-TMS
623(300)	Completed	Completed	except E-TMS
993(456)	Completed	Completed	except E-TAB & E-TMS
1585(621)	Completed	Completed	except E-TAB & E-TMS
1586(622)	Complted	Completed	except E-TAB & E-TMS
1799(676)	Complted	except S-TMS & E-TAB	except E-TAB,E-TMS & S-TMS

Several previously unencountered and currently unidentified components have been detected in some samples, for example, two new components in the spectra of several of the lung cancer samples. Because these may be artifacts (e.g., drugs or drug metabolites not covered in our current libraries) any more definitive statement must await the patient histories.

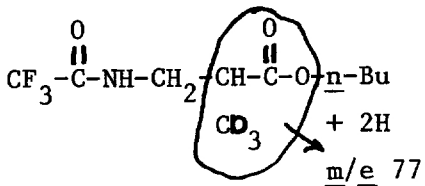
Results of the past 18 months have led us to pursue two lines of activity for the remaining period of this grant, in addition to finishing the few analyses which remain in the preliminary collection and analysis of data (Table II).

1. Precise quantitation of BAIB. We can evaluate the utility of BAIB as a potential diagnostic marker only when several additional experiments are completed. The first is precise quantitation of BAIB in sets of samples where it was detected in some patients at high levels and comparison of these data with approximate controls. To this end, we have begun refining our current method for the quantitation of BAIB in urine by mass fragmentography ("The Quantitation of β-Aminoisobutyric Acid in Urine by Mass Fragmentography", W. E. Pereira, R. E. Summons, W. E. Reynolds, T. C. Rindfleisch, and A. M. Duffield, Clinica Chimica Acta 49, 401-406, 1973). This method, utilizing a Tabsorb GC column and the GC/MS/computer to monitor masses 153 and 182 of the N-TFA-O-n-butyl ester of BAIB, suffers from interference of masses of isoleucine, which elutes from the GC with the same relative retention index.

We have determined (by high resolution mass spectrometry provided by our DENDRAL collaborators) that the mass 74 ion of BAIB-N-TFA-O-n-butyl ester is unique to BAIB, possesses composition $C_3H_6O_2$ and most probably represents the portion of the molecule circled in structure 1. We will synthesize the deuterium labeled analog 2 and monitor m/e 74 with respect to m/e 77 (from the same fragmentation of 2; 2 added in known quantities) to quantitate BAIB.



1



2

2. Detailed Intercomparison of Samples. We must examine the excretion profiles of the patients in more detail. Manual examination can catch only the grosser abnormalities, because there are far too many data for more careful comparison of results of one sample with a previous history of results. We plan to develop the computer programs necessary to automate this procedure. The concepts are straightforward and several of the programs are largely modifications of existing software.

The goal of this effort is to provide the chemist a summary report on the similarities and dissimilarities among the organic constituents of urines of selected sets of patients. It must be flexible enough to compare patients with the same or different cancers or either with controls, and to compare a patient or patients with a more comprehensive history of components detected in any previous analysis independent of knowledge of the structure of the component.

Briefly, these programs will begin after CLEANUP and library search are completed. The relative retention index of each component, determined from hydrocarbon standards added to the data, is calculated. Each set of spectra is then compared to a "local" library of spectra (from one or more related patients or a more comprehensive set) where the matching criteria are retention indexes and similarity of spectra. The hydrocarbon standards provide a means for semi-quantitative estimation of the relative amounts of each component.

X1000 ETE NANCY'S #344 BPH
ET303 01-DEC-75

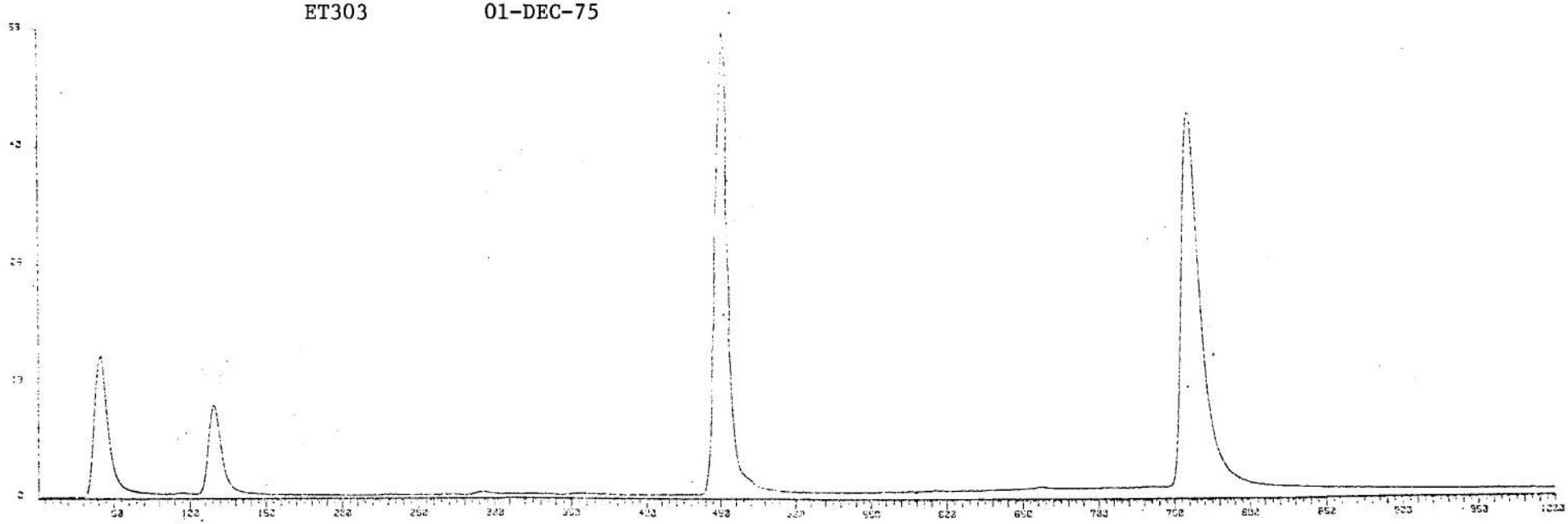


FIGURE I

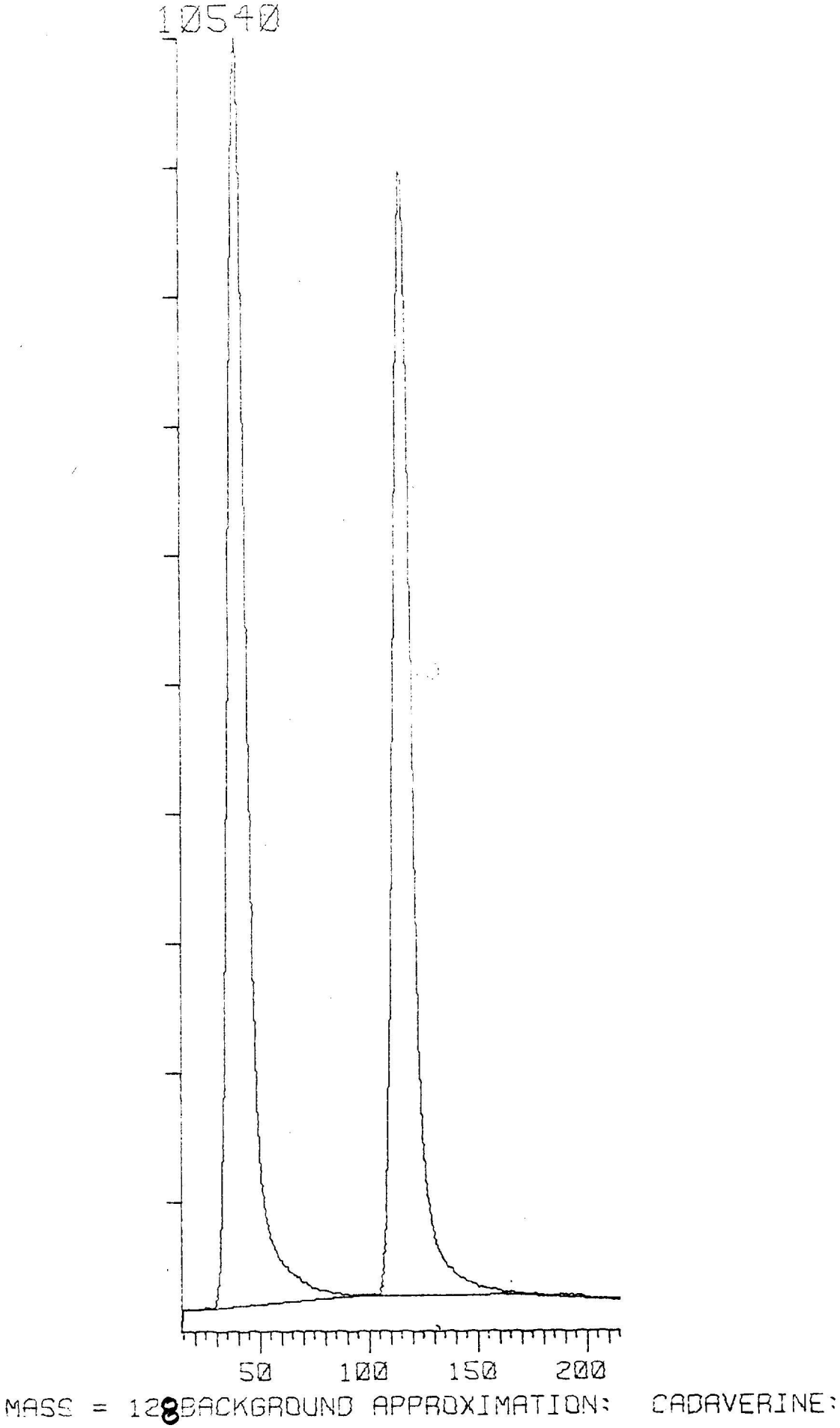

10540
50
100
150
200
MASS = 128
BACKGROUND APPROXIMATION:
CADAVERINE:

FIGURE II


10328
50
100
150
200
MASS = 128 THRESHOLDED PEAKS & AREAS: CADAVERINE:

FIGURE III

CALIBRATION CURVE : CADAVERINE.

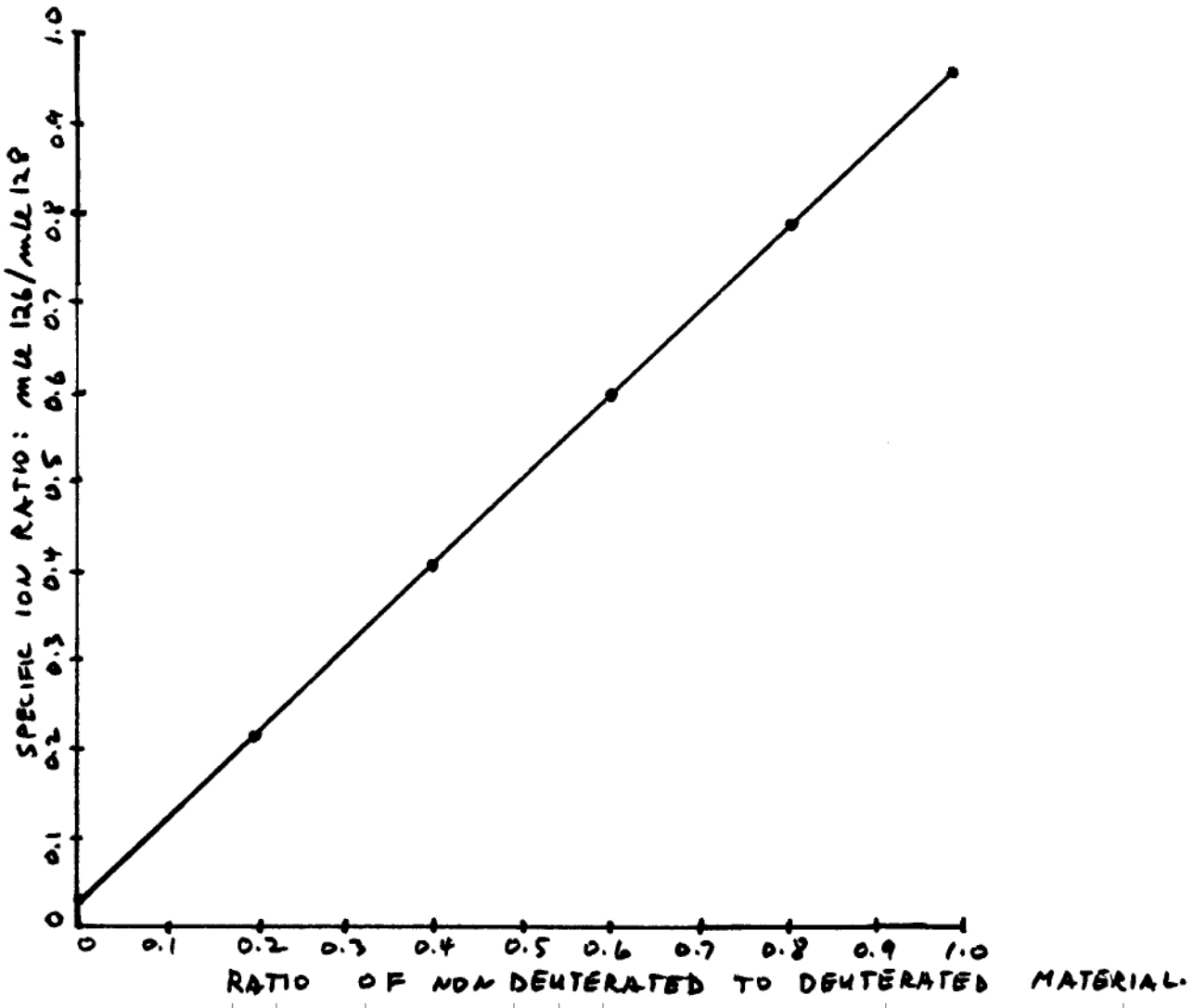


FIGURE IV