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Tryptophan Metabolism by Carcinoid Tumors

by

**Alvin Jay Greenberg
B.S. University of Illinois 1969**

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Pharmaceutical Chemistry

in the

GRADUATE DIVISION

(San Francisco)

of the

UNIVERSITY OF CALIFORNIA



Abstract

The in vivo and in vitro metabolism of tryptophan by carcinoid tumors was investigated in order to ascertain the complete metabolic profile of this amino acid. Techniques were applied to quantify as well as identify metabolites produced. Knowledge of the tryptophan metabolism by the tumor may aid diagnosis of this disease and treatment of the patient involved and serve to increase our general knowledge of the biochemical potential of carcinoid tumors.

Carcinoid tumors are usually malignant carcinomas arising from the argentaffin cells throughout the lung and gastrointestinal tract and, depending upon the primary site, metastasize readily, most commonly to the liver. It is at this stage that the heretofore undiagnosable carcinoid tumor is most commonly diagnosed as a result of clinical manifestations known as the carcinoid syndrome. Associated with this syndrome are large amounts of pharmacologically active substances such as kallikrein, bradykinin, histamine and 5-hydroxytryptamine present in the circulation of carcinoid patients. This increase in 5-hydroxytryptamine and the excessive excretion of its principal metabolite, 5-hydroxyindoleacetic acid (5-HIAA) constitute the biochemical hallmark of this disease. Recent investigations have shown that some patients with carcinoid tumors exhibit low urinary 5-HIAA levels and/or elevated levels of other indolic metabolites, including 5-hydroxytryptophan and indoleacetic acid (IAA). It is the deviation from the classical metabolism of tryptophan to 5-HIAA by carcinoid patients that is the subject of this investigation.

A new analytical technique employing chemical ionization mass spectrometry (C.I.M.S.) was developed to identify and quantify metabolites excreted. C.I.M.S. involves lower energy than electron impact mass spectrometry thus producing very simplified spectra which include a molecular ion for even the most unstable compounds. High resolution continuous ion monitoring of two ion currents was used to determine IAA/5-HIAA ratios present in the urines of patients. The synthesis of several stable-isotope labeled internal standards, including d_4 -tryptophol, d_4 -5-hydroxytryptophol and d_7 -IAA, enabled quantification of these compounds by C.I.M.S.

After screening over twelve carcinoid patient urines by thin layer chromatography, gas chromatography and C.I.M.S., only one new metabolite of tryptophan was identified. One patient was found to have excreted indole-3-pyruvic acid. The C.I.M.S. continuous ion monitor was useful in initial screening of indolic compounds present in the urine but was not sufficiently accurate in its present stage of development to use as a method for quantifying the compounds under scrutiny. The C.I.M.S. internal standard assay was useful in determining tryptophol, 5-hydroxytryptophol and IAA urinary excretion in two patients and in a Rhesus monkey.

Studies on the possible pharmacological activity of indoleacrylic acid demonstrated that this compound did not produce hypoglycemia in rats or rabbits.

Experiments using deuterium labeled tryptophan (in the 5-position) in a loading dose administered to animals demonstrated that a ratio of greater than 50:50 labeled to unlabeled must be used to prevent excessive

The first step in the process of identifying a problem is to define the problem clearly. This involves identifying the symptoms and the underlying causes of the problem. Once the problem is defined, the next step is to gather information about the problem. This can be done through a variety of methods, including interviews, surveys, and observations. The information gathered should be analyzed to identify the root cause of the problem. Once the root cause is identified, the next step is to develop a plan to address the problem. This plan should be based on the information gathered and should take into account the resources available and the time constraints. The plan should be implemented and the results should be monitored to ensure that the problem is resolved.

Once the problem is resolved, it is important to evaluate the effectiveness of the solution. This can be done by comparing the results of the solution to the original problem. If the problem has been resolved, the solution is effective. If the problem has not been resolved, the solution is ineffective. In this case, the process should be repeated to identify the root cause of the problem and develop a new plan to address it.

dilution of labeled compound by endogenous tryptophan and endogenous tryptophan metabolites. The large amount of labeled tryptophan unaccounted for as metabolites raised the question of patient safety when administering large amounts of stable-isotope labeled amino acids.

In vitro studies on carcinoid tumor tissue homogenates incubated with ^{14}C -L-tryptophan were inconclusive due to the questionable viability of tumor tissue obtained 15 hr post mortum. An attempt to culture carcinoid cells failed.

An attempt to synthesize and isolate a suspected metabolite, 5-hydroxyindole-3-pyruvic acid was unsuccessful. Synthesis was achieved but isolation and complete characterization was not possible. Another suspected metabolite, 5-methoxyindole-3-acrylic acid was synthesized, isolated and characterized successfully, the first reported preparation of this compound.

To Me

Acknowledgments

I would like to thank the following people who have been with me during the past six years:

To Roger Ketcham for having faith and confidence in me when others did not;

To Hibbard Williams for guidance and instruction;

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To my friends in the School of Pharmacy office for their humor and support;

To my father, sister and brother for their love;

and to Paulann for helping me learn how to feel, something my doctoral education did not do.

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QUESTION

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Year	Number of people
2010	120
2011	150
2012	180
2013	210
2014	240

Find the mean.

2. The following table shows the number of people who attended a concert in each of the five years from 2010 to 2014. The number of people who attended the concert in each year is given in the table below.

Year	Number of people
2010	120
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Find the mean.

4. The following table shows the number of people who attended a concert in each of the five years from 2010 to 2014. The number of people who attended the concert in each year is given in the table below.

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1. The first step in the process of identifying a problem is to recognize that a problem exists. This is often done by comparing current performance with a desired state or goal. For example, a manager might notice that sales are declining or that customer satisfaction is low. Once a problem is identified, the next step is to define it more precisely. This involves determining the scope of the problem, its causes, and its effects. For instance, a manager might define a problem as "a 10% decline in sales over the last quarter, primarily due to a loss of market share in the competitive market." The third step is to analyze the problem. This involves gathering data, identifying key factors, and determining the underlying causes. For example, a manager might analyze sales data to identify trends, compare performance with competitors, and identify areas where the company is losing market share. The fourth step is to generate potential solutions. This involves brainstorming ideas, evaluating alternatives, and selecting the most promising options. For instance, a manager might generate solutions such as "implementing a new marketing strategy," "improving customer service," or "reducing prices." The fifth and final step is to implement the chosen solution and monitor its progress. This involves developing a plan, allocating resources, and tracking performance over time. For example, a manager might implement a new marketing strategy and monitor sales and market share over the next quarter.

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1. The first step in the process of identifying a problem is to recognize that a problem exists. This is often done by comparing current performance with a desired state or goal. For example, a manager might notice that sales are declining or that customer satisfaction is low. Once a problem is identified, the next step is to define it more precisely. This involves determining the scope of the problem, its causes, and its effects. For instance, a manager might define a sales decline as a 10% drop in revenue over the last quarter, caused by a decrease in the number of new customers and a loss of existing customers. The third step is to analyze the problem. This involves gathering data, identifying patterns, and testing hypotheses. For example, a manager might analyze sales data to see if there is a seasonal trend or if the decline is more pronounced in certain regions. The fourth step is to generate alternative solutions. This involves brainstorming ideas and evaluating their potential benefits and costs. For example, a manager might consider increasing marketing efforts, offering discounts, or improving customer service. The fifth step is to select a solution. This involves choosing the most promising alternative based on the available information. The final step is to implement the solution and monitor its progress. This involves putting the chosen solution into action and tracking its performance over time to see if it effectively addresses the problem.

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PART I

INTRODUCTION

Introduction

I

The objective of this research was to investigate the in vivo and in vitro metabolism of tryptophan by carcinoid tumors in order to ascertain the complete metabolic profile of this amino acid. Techniques were applied to quantify as well as identify any metabolites produced. Knowledge of the tryptophan metabolism by the tumor may aid diagnosis of this disease and treatment of the patient involved and serve to increase our general knowledge of the biochemical potential of carcinoid tumors.

Carcinoid tumors are usually malignant carcinomas arising from the argentaffin cells throughout the lung and gastrointestinal tract. These tumors are most often found in the appendix, ileum, stomach, rectum, colon and bronchus and rarely in the pancreas and biliary tract. The tumor comprises 0.05% to 0.20% of all neoplasms (1). These tumors are often very small in size and, depending upon the primary site, metastasize readily, most commonly to the liver. It is usually at this stage that the heretofore undiagnosable carcinoid tumor is most commonly diagnosed as a result of clinical manifestations known as the carcinoid syndrome (2). This syndrome or carcinoid spectrum, as Melmon and Sjoerdsma refer to it, consists of episodic flushing, diarrhea, bronchoconstriction and hypotension all of which may have a duration of several minutes to several days (3,4). Of 3,718 cases of abdominal carcinoid tumors collected by Wilson (5), only 136 patients (3.7 per cent) had symptomatic endocrinologic

activity and were classified as having the carcinoid syndrome. The tumors producing carcinoid symptoms are mostly from the jejunum and ileum followed in frequency by the stomach, appendix, colon and duodenum. In gastrointestinal carcinoids, the presence of the carcinoid syndrome almost always signifies metastatic disease to the liver with subsequent impairment of hepatic degradation of pharmacologically active substances produced by the tumor. On the other hand, the venous drainage of bronchial tumors is systemic and bypasses the liver; hence, early disease without metastases may produce symptoms.

This clinical syndrome is associated with numerous biochemical abnormalities. Large amounts of pharmacologically active substances such as kallikrein, bradykinin, histamine, and 5-hydroxytryptamine (5-HT), which may or may not be secreted by the tumor, have been detected in the circulation of carcinoid patients (6,7). This increase in serotonin in the blood and excessive excretion of the metabolites of serotonin, principally 5-hydroxyindoleacetic acid (5-HIAA) constitute the biochemical hallmark of this disease.

Considerable interest has been generated in the last several years concerning the biochemical profile of this unique tumor. E.D. Williams and M. Sandler classified carcinoid tumors using morphological, histochemical and biochemical bases (8). They found that ileal carcinoids arise from the embryonic midgut, frequently metastasize to the liver and produce a definite carcinoid syndrome. An ileal tumor also contains a high concentration of 5-HT and patients usually exhibit the classic pattern of elevated urinary

The first part of the document discusses the importance of maintaining accurate records of all transactions. It emphasizes that proper record-keeping is essential for the success of any business and for the protection of the interests of all parties involved. The document outlines the various methods and systems that can be used to ensure the accuracy and reliability of financial records.

The second part of the document provides a detailed overview of the accounting process, from the initial recording of transactions to the final preparation of financial statements. It covers the various steps involved in the accounting cycle, including the identification of transactions, the recording of those transactions in the accounting system, and the subsequent analysis and reporting of the results.

The final part of the document discusses the role of the accountant in the business and the importance of maintaining high ethical standards. It emphasizes that accountants have a responsibility to provide accurate and reliable information to their clients and to the public, and that they must always act in the best interests of their clients and the public.

5-HIAA. Bronchial, pancreatic and gastric carcinoids arise from the embryonic foregut and metastasize not only to the liver but to the skin and bone as well. These tumors excrete 5-hydroxytryptophan (5-HTP) as well as 5-HIAA into the urine. In addition, the gastric tumor often lacks the decarboxylase enzyme and large amounts of 5-HTP are excreted along with the 5-HIAA. Patients with gastric carcinoids frequently excrete large amounts of histamine. Some patients exhibit low urinary 5-HIAA levels and/or elevated indoleacetic acid (IAA) levels. It is the deviation from the classical metabolism of tryptophan to 5-HIAA by carcinoid patients that is the subject of this investigation.

The first investigation of the role of serotonin in the carcinoid syndrome was by Thorson and associates in 1954, based on review of past reports (9,10,11). Serotonin was implicated in pulmonary hypertension, bronchoconstriction, increased intestinal peristalsis, and right-sided valvular lesions. On the basis of those observations, Waldenstrom and Ljungberg noted elevated serotonin concentrations in the serum of carcinoid patients and determined that urinary concentrations of 5-HIAA were also elevated (12). Further investigation of in vitro tryptophan metabolism by carcinoid tumors demonstrated the presence of 5-HTP as well as 5-HIAA (13). It was at this stage that investigation of tryptophan metabolism rested until variations of the classic carcinoid syndrome led to the concept of the carcinoid spectrum suggested by Melmon, et al in 1968 (3). Associated with these symptomatic variations were variations in urinary indole acids. A distinctive biochemical potential has been suggested for carcinoid tumors of different primary sites. Very recent work by H. Williams,

et al., has shown that carcinoid tumors do exist without the patient exhibiting the biochemical hallmark of elevated urinary 5-HIAA (14). Williams and associates demonstrated through the use of gas-liquid phase chromatography (GLPC) and mass spectral analysis that other indolic acids are present in the urine of carcinoid patients. Studying a total of eleven carcinoid patients, it was determined that three of the patients had either normal or slightly elevated 5-HIAA urinary levels yet had biopsy proven carcinoid tumors and exhibited several symptoms of the carcinoid syndrome. Studies in these three patients indicated that determination of the pattern of various indolic metabolites of tryptophan after tryptophan loading allowed specific metabolite characterization of these patients and classification of the other types of the carcinoid syndrome into four distinct types. These findings indicate the biochemical diversity of the carcinoid tumor and demonstrate that the diagnosis cannot be based exclusively on the finding of elevated urinary 5-HIAA levels. This idea is also supported by Ureles (15) in a review of diagnosis and treatment of carcinoid tumors and by Kaplan, Jaffe and Peskin (16) who describe a new provocative test for the diagnosis of the carcinoid syndrome, employing calcium infusion, as superior to the frequently errant results of blood serotonin and urinary 5-HIAA determinations.

Investigation of the effects of altered tryptophan metabolism was also conducted by Sjoerdsma and Udenfriend to shed light on the etiology of the pellagra like syndrome and hypoalbuminemia that may develop in carcinoid patients (17,18) Only 1 per cent of dietary tryptophan is utilized for serotonin biosynthesis in normal persons

while 99 per cent is available for incorporation into protein and metabolism to niacin via the kynurenine pathway. In carcinoid patients, on the other hand, as much as 60 per cent is directed into the serotonin pathway which may leave a deficient amount for production of protein and niacin. Sjoerdsma believed that the excess serotonin might be responsible for these deficiency states. A correlation does exist between the deficiency of nicotinic acid in pellagra patients and levels of serotonin in the serum and of 5-HIAA in the urine (19). However, recent thinking casts doubt that deficiency syndromes associated with carcinoid tumors can be attributed to this massive shunting of tryptophan from normal pathways to production of serotonin (20). There is doubt that a clinical deficiency of niacin could result when 40 percent of the tryptophan pool remains available for its synthesis. The possibility that tryptophan is directed to other pathways is clearly indicated.

One of the purposes of this research was to develop new techniques for efficient and unambiguous identification of in vivo and in vitro metabolites of tryptophan. Previous methods of detection of serum and urinary acids and bases including serotonin and 5-HIAA include thin layer chromatography, spectrofluorometric assay and gas-liquid-partition chromatographic analysis (G.L.P.C.) after suitable derivatization (21,22,23,24,25,26). These techniques often lead to ambiguous and misleading values, resulting from "false positives" due to interference from other compounds of similar structure and chemical reactivity. These techniques were augmented with the use of electron impact mass spectrometry (E.I.M.S.)

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The study was conducted over a period of six months, during which time a large amount of data was collected and analyzed. The results of the study are presented in the following sections. The first section discusses the overall findings, while the second section provides a more detailed analysis of the data. The third section discusses the implications of the findings for practice and policy. The final section concludes with a summary of the study and a list of references.

to determine the molecular formula of derivatized metabolites eluted from G.L.P.C. or T.L.C. (14,27). More recently, the analytical techniques of combined gas chromatography (GC), mass spectrometry (MS), and mass fragmentography (MF) with computer analysis have made possible the measurement of endogenous amines including serotonin in the picomole range (28,29,30). I have now developed techniques employing chemical ionization mass spectrometry (C.I.M.S.). The advantages of C.I.M.S. over electron impact M.S. or M.F. are elimination of the necessity to derivatize the compounds in question to afford sufficient volatility and decreased fragmentation of the molecule that allows one to focus attention on the parent peak of the compound rather than on the fragments. Parent peaks and decreased fragmentation are usually achieved for even the most unstable compounds (31,32). The spectrum is simplified and essentially consists of only a parent peak representing the compound's molecular weight plus one (M+1) as the mechanism of C.I.M.S. involves protonation of the compound. Thus, a spectrum of a mixture of compounds would contain only one major mass peak (M+1) for each compound present and very few minor peaks representing fragmentation. The ability to see the parent peaks of compounds of biologic interest and thus to know their molecular weights constitutes an important step in the identification of a myriad of metabolites. Isobutane C.I. was employed by Milne, Fales and Axenrod for the rapid identification of drugs taken in overdose quantities by patient (33). Identification of compounds ranging from phenacetin and aspirin to oxycodone and Amytal was achieved by simple chloroform extraction of serum or gastric contents and direct C.I.M.S. without derivatization. This was a remarkable demonstration of the utility of this procedure.

In order to more fully realize the potential of C.I.M.S., the use of a "label" of one form or another that one could detect in the mass spectrum would serve to rapidly identify any compound that is a derivative of the tryptophan administered to a patient. An oral load of L-tryptophan has been employed by many researchers including Williams and in our own experiments as a useful means of allowing detection of abnormal metabolism which might otherwise go undetected (14, 34, 35).

Previous work employing the stable isotopes ^{13}C and ^2H showed good potential application in the study of drug metabolism (36, 37, 38, 39). One possible choice of label is mon-deuterated tryptophan. Following the lead of previous research, administration to carcinoid patients of a tryptophan load consisting of 50% mono-deuterated L-TP and 50% normal L-TP should yield urinary metabolites of molecular weight M (from normal L-TP) and M+1 (from mono-deuterated L-TP) in a ratio of 1:1. Thus, a C.I.M.S. of a urine sample would show doublets corresponding to M+1 and M+2 for all metabolites derived from the administered 50% labeled tryptophan. Another choice would be ^{13}C incorporated somewhere in the molecule. The use of two stable isotopes, two deuterium or one ^{13}C and one deuterium would result in parent peaks of M+1 and M+3. A doublet separated by two mass units may prove to be better in that interference from the natural abundance of ^{13}C would be eliminated.

Although the administration of 50% labeled compound may be suitable for exogenous compounds such as drugs, the body pool of an endogenous compound such as tryptophan is so large that the label becomes too

dilute to detect. I have found this to be true in my experiments with rats, rabbits, and Rhesus monkeys employing 50% 5-²H-DL-tryptophan administered with normal DL-tryptophan (100mg/kg body weight) and now realize that administration of 100% labeled L-tryptophan would be a more satisfactory technique.

The use of a stable isotope rather than a radioisotope has several advantages. The stable isotope can be detected by mass spectrometry and thus its identity can be more easily determined and stable isotopes pose no long term radiation hazard to a patient. Administration of a radioactive amino acid would result in incorporation of radioactivity into protein. It is felt that the long-term presence of stable isotopes in the body is significantly less hazardous than the presence of radioisotopes, although the possible toxicity of stable isotopically labeled compounds is still to be completely determined (38,39)

It is also possible to quantify all metabolites derived from tryptophan with C.I.M.S. employing stable isotopically labeled metabolites as internal standards. The relative peak heights of the standard and metabolite are a direct measure of the amount of metabolite present. I believe that this method is superior to the standard spectrometric assays now in use for 5-HT, 5-HIAA and IAA determination (21,22,23,25).

Thus, the specific aims of this investigation were:

1. To synthesize "suspected" metabolites of tryptophan to serve as authentic standards.

2. To prepare stable-isotope labeled metabolites to be used in a C.I.M.S. assay.
3. To survey the in vivo metabolism of tryptophan by patients with carcinoid tumor.
4. To correlate the quantities and identities of metabolites with the primary site of the tumor.
5. To synthesize stable-isotope labeled tryptophan.
6. To develop a culture of carcinoid tissue or cells in order to more fully realize the potential of in vitro experimentation. Previously, growth of carcinoid tumors in tissue culture has been demonstrated (40). A cell culture would immensely facilitate not only the study of the metabolism of tryptophan by the tumor in vitro, but also the study of tryptophan uptake, pooling and turnover by the tumor cells. This would help to develop a more thorough understanding of the relationship between tryptophan and the tumor and also allow for the study of drugs which might block uptake or metabolism of tryptophan.
7. To study the in vitro metabolism of tryptophan by carcinoid tumors and drugs which may effect its metabolism.
8. To determine any possible pharmacologic activity of selected metabolites.

Any correlation between differences in amounts and types of metabolites produced with the presence of carcinoid tumor may hopefully aid in further classification of these tumors such that earlier and better diagnosis is possible. Furthermore, a better understanding

the fact that the system is not a simple linear system, but a complex system with many interacting components. The system is a complex system because it is composed of many interacting components, each of which has its own set of properties and behaviors. The system is not a simple linear system because the interactions between the components are not linear, and the system as a whole exhibits emergent properties that are not predictable from the individual components. The system is a complex system because it is composed of many interacting components, each of which has its own set of properties and behaviors. The system is not a simple linear system because the interactions between the components are not linear, and the system as a whole exhibits emergent properties that are not predictable from the individual components.

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of the metabolism of this important amino acid may serve to focus attention on feasible mediators of certain aspects of the clinical syndrome. Characteristically, patients with carcinoid tumors have a protracted disease course during which the patient suffers considerable distress from the acute episodes that are believed to be secondary to the elaboration of pharmacologically active substances from the tumor. Treatment with steroids, phenothiazines, and serotonin antagonists produce dramatic relief in some patients and no effect in others (41,42,43). A more complete understanding of the disease could allow for more specific, selective and effective therapy.

The recent work by Williams (15) and current investigations in our own laboratory indicate that a number of previously undiscovered indole acids (and presumably their precursor amines) may be formed by patients with carcinoid tumor. Furthermore, the quantity of indole excretion may lead to diagnosis of this disease in patients with normal excretion of 5-HIAA and that the variation of the metabolites excreted may provide a simple biochemical means to help establish the site of the primary tumor and serve to explain the pathogenesis of some symptoms.

Ancillary to my primary rationale for the continued investigation of tryptophan metabolism by carcinoid tumors is the possible application of our analytical techniques to other disease states where abnormal tryptophan metabolites of the kynurenine pathway, principally 3-hydroxykynurenine, xanthurenic acid and 3-hydroxyanthranilic acid, have been noted in patients with carcinoma of the breast (44,45,46,47,48). In carcinoma of the bladder, the urinary excretion levels of certain tryptophan metabolites may be increased (49,50,51). Certainly, my

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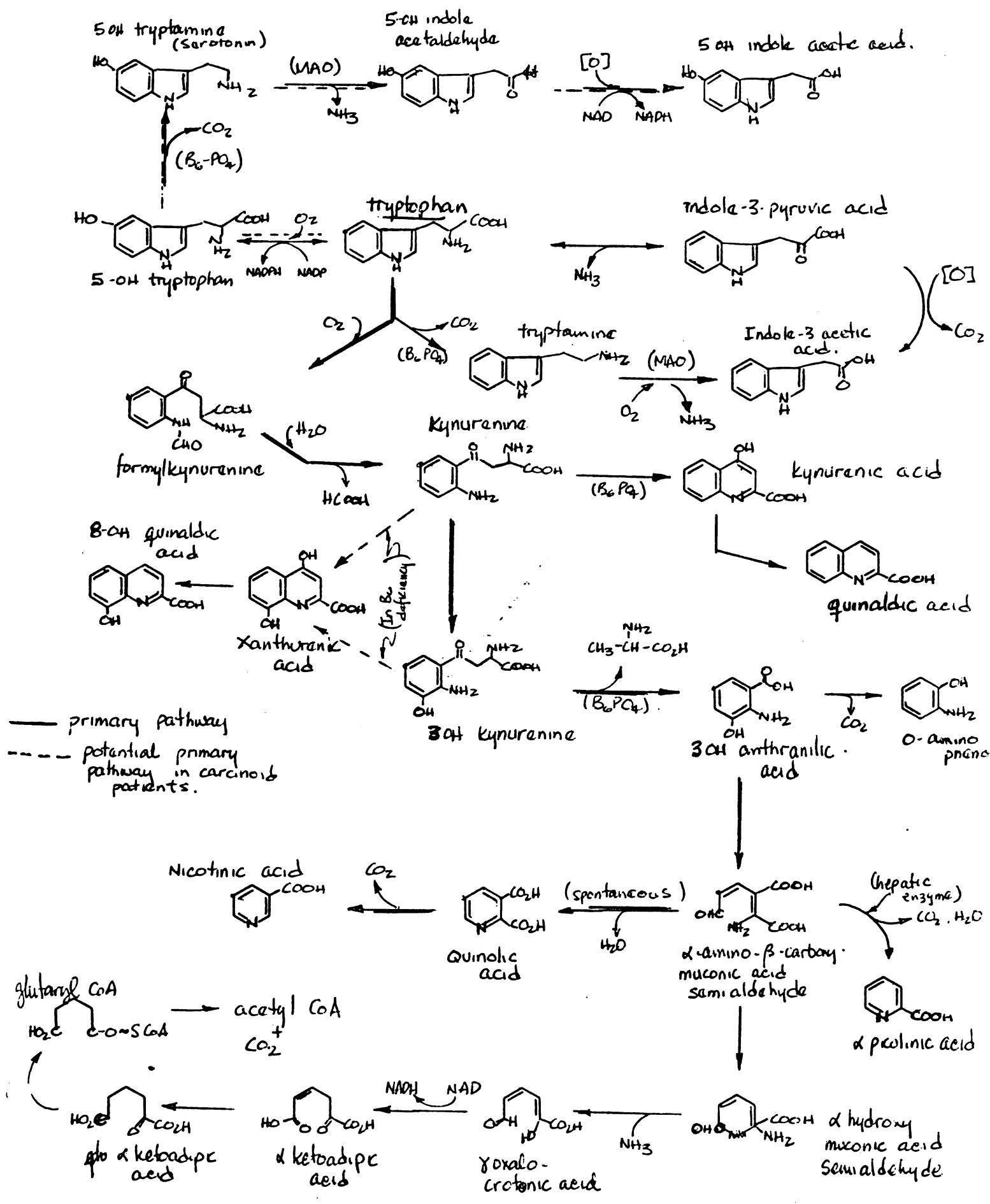
In addition, the document outlines the various methods and tools used for data collection and analysis. It mentions the use of spreadsheets, databases, and specialized software to manage large volumes of information. The text also discusses the importance of data security and privacy, particularly in the context of handling sensitive financial information.

The final section of the document provides a summary of the key findings and conclusions. It reiterates the importance of consistent and accurate record-keeping for the overall success of the organization. The text also offers some practical recommendations for improving the efficiency and effectiveness of the financial reporting process.

research into carcinoid tumor metabolism might aid other researchers in obtaining a better understanding of the metabolism of this important amino acid associated with other forms of cancer. Finally, there has been increased interest in the possible role that tryptophan metabolites might play in the hypoglycemia associated with neoplasia (52). A possible diabetogenic role for tryptophan and several of its metabolites including 3-hydroxykynurenine, 3-hydroxyanthranilic acid, quinaldic acid, indole-3-propionic acid, indole-3-butyric acid and indole-3-acetic acid (IAA) had been suggested (52,53,54). I felt that it was desirable to explore the possibility of other metabolites possessing this activity.

In summary, the possible gain in formation from this research could conceivably benefit patients inflicted with carcinoid tumor and lay the basis for future work in other areas of tryptophan metabolism by other neoplasia.

TRYPHTOPHAN METABOLISM



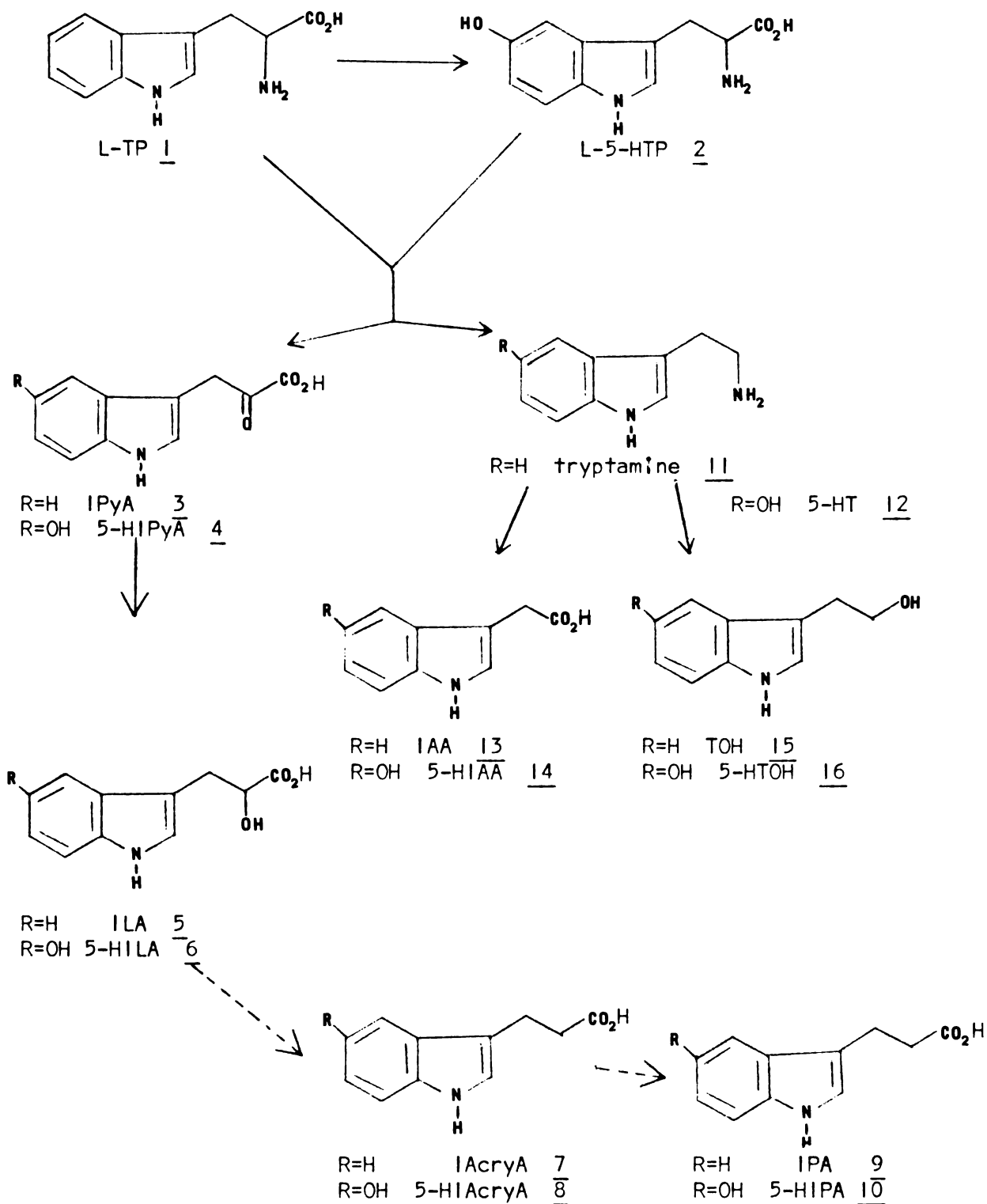


Fig. 2

Tryptophan metabolism to indole and 5-hydroxyindole metabolites.

PART II

CHEMICAL

A. Synthesis of Possible Metabolites

5-Hydroxyindolepyruvic Acid

Introduction

The compound 5-hydroxyindole-3-pyruvic acid (5-HIPyA 4) is of particular interest as a suspected metabolite in persons with carcinoid tumor. Active oxidative deamination of aromatic amino acids to α -keto acids is well known and brain transaminase(s) have been partially purified (55,56,57,58,59,60). In addition, administration of D-5-hydroxytryptophan to test animals results in an increase in serotonin production leading several authors to suggest that the D-isomer is oxidatively deaminated to the α -keto acid, transaminated to the L-amino acid and subsequently decarboxylated to the amine (61, 62,63). Because some carcinoid tumors have high levels of tryptophan hydroxylase activity and some patients excrete large amounts of 5-hydroxylated indole compounds in the urine, it seems reasonable to suspect that some 5-hydroxytryptophan may be deaminated to 5-hydroxyindole-3-pyruvic acid. Millard and Gal (64). presented indirect evidence for the presence of an alternate pathway for 5-hydroxyindole metabolism including 5-hydroxyindole-3-pyruvic acid. Although they claim to have isolated 5-hydroxyindolepyruvic acid as its 2,4-dinitrophenylhydrazone, from both a 30,000 xg supernatant prepared from rat brain homogenates and from an incubation of ^{14}C -DL-5-hydroxytryptophan or ^{14}C -L-tryptophan with *Crotalus adamanteus* venom (rich in aromatic aminotransferase), they did not report any data other than comparative thin layer chromatography to substantiate their claim. No synthesis of 5-HIPyA or its 2,4-dinitrophenylhydrazone was reported. Nevertheless, Millard and

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Gal succeeded in pointing out the necessity to synthesize an authentic sample in order to more fully assess the contribution 5-hydroxyindole-pyruvic acid may have to a bypass of cerebral 5-hydroxyindole metabolism and to carcinoid tryptophan metabolism.

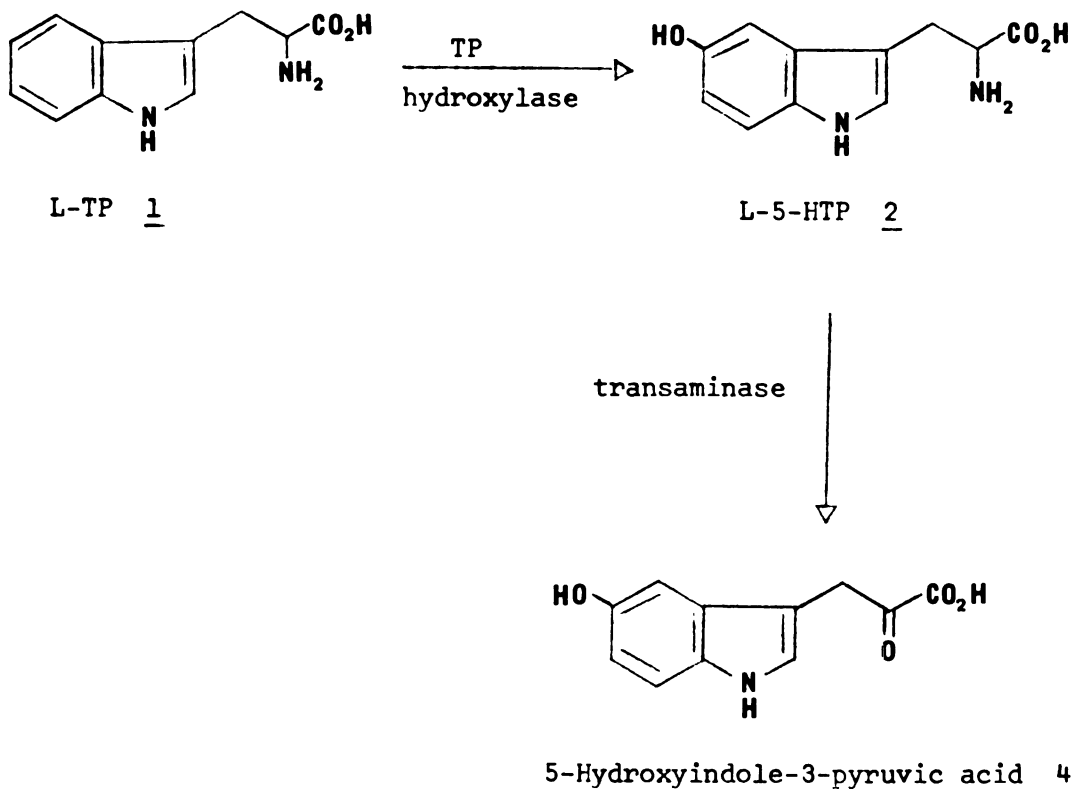
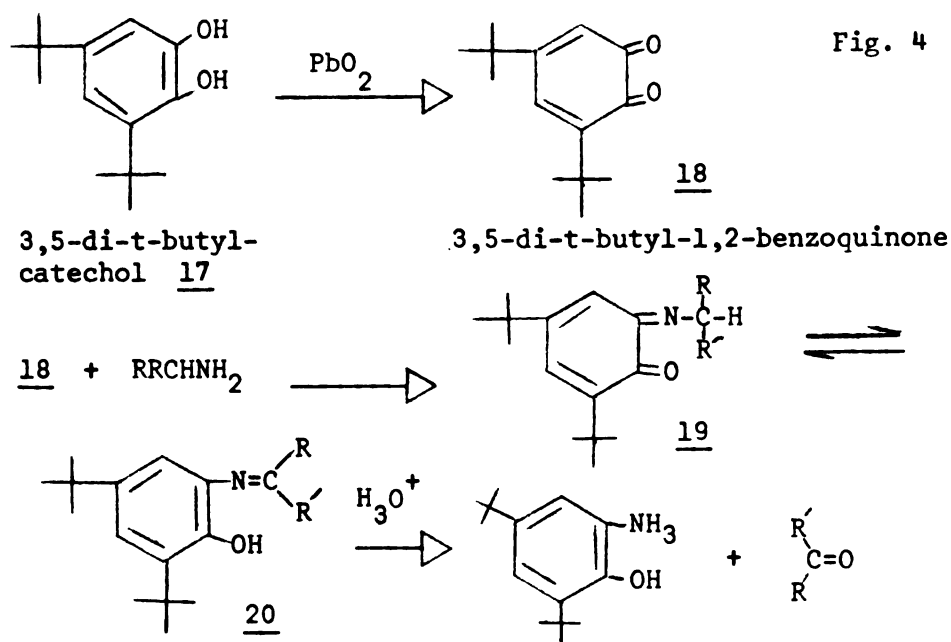


Fig. 3 Metabolic formation of 5-Hydroxyindole-3-pyruvic acid.

Synthetic Oxidative Deamination: An Attempt to Prepare α -Keto
Acids From α -Amino Acids

An early attempt to prepare the suspected metabolite, 5-hydroxyindole-3-pyruvic acid, involved the use of a quinone reagent employed by Corey *et al* (65) in the conversion of primary amines to ketones. This reagent, 3,5-di-*t*-butyl-1,2-benzoquinone (18) reacts with an amine to form a Schiff base intermediate at position one of the quinone (19). The two position is sterically hindered by the neighboring *t*-butyl group and reaction occurs only at position one. Subsequent tautomerization to the aromatic tautomer 20 followed by mild acid hydrolysis of the imine produces ketones in very high yields.



The synthesis of the ortho quinone from 3,5-di-*t*-butylcatechol (17) was achieved according to the procedure of Flaig *et al* (66) with the modification of recrystallization from EtOH. Repetition of Corey's synthesis of cyclohexanone and acetophenone from cyclohexylamine and

Section 1: Introduction

The purpose of this document is to provide a comprehensive overview of the project's objectives, scope, and timeline. The project aims to develop a new software application that will streamline the workflow of our organization. The scope of the project includes the design, development, testing, and deployment of the application. The timeline for the project is estimated to be 12 weeks, starting from the beginning of the month and ending by the end of the month. The project will be managed by a dedicated team of developers, designers, and testers. The project will be divided into several phases, including requirements gathering, design, development, testing, and deployment. The project will be completed by the end of the month, and the application will be available for use by all users. The project will be a significant milestone for our organization, and we are confident that it will greatly improve our efficiency and productivity.

Section 2: Project Objectives

The primary objective of this project is to create a user-friendly software application that meets the needs of our organization. The application should be able to handle all the tasks currently performed manually, and it should be able to do so in a more efficient and accurate manner. The application should also be able to integrate with our existing systems, and it should be able to provide real-time data and reporting. The project objectives are to: 1) Define the requirements for the application, 2) Design the application architecture, 3) Develop the application code, 4) Test the application for bugs and performance, and 5) Deploy the application to the production environment. The project will be completed by the end of the month, and the application will be available for use by all users.

Section 3: Project Scope

The project scope includes the design, development, testing, and deployment of the application. The project will be completed by the end of the month, and the application will be available for use by all users. The project will be a significant milestone for our organization, and we are confident that it will greatly improve our efficiency and productivity.

α -phenylethyl amine respectively gave the two ketones in yields of 74 and 54%.

In order to prepare α -keto acids from α -amino acids, it was necessary to use the esters of the amino acids. Amino acids exist as the zwitter ion in solution and it is doubtful if the protonated amine would function as a nucleophile to form the initial Schiff base. The methyl esters of tyrosine and tryptophan are available commercially and the methyl ester of 5-hydroxytryptophan was prepared employing thionyl chloride in methanol (fig. 5). It is not clear whether nucleophilic attack by the carboxylate function of the amino acid occurs on a methoxysulfonyl chloride species 21 or on a dimethoxysulfone species 22, but both will yield the anhydride 23 which when attacked by methanol will give the desired methyl ester 24. This procedure is particularly useful in that no reaction past the anhydride stage is possible with the phenolic hydroxy and thus no methyl ether is obtained.

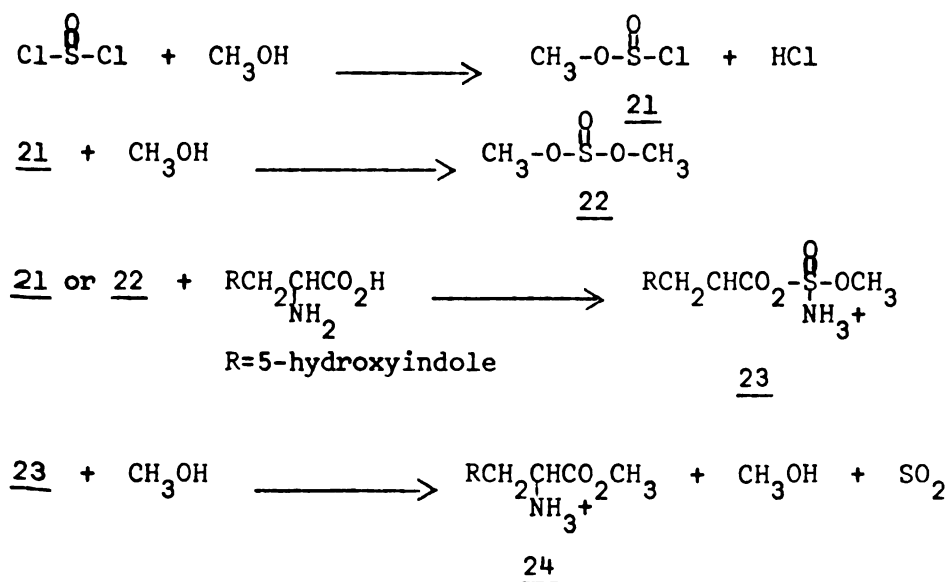


Fig. 5 Synthesis of 5-hydroxytryptophan methyl ester.

The oxidative deamination reaction was not successful with α -amino acids. Tyrosine methyl ester was used as a model because of the availability of p-hydroxyphenylpyruvic acid and tryptophan methyl ester was tried when a sample of indolepyruvic acid was obtained. In both cases, it was impossible to detect or isolate an α -keto acid. Some starting material was recovered, however. The reaction with 5-hydroxytryptophan methyl ester was also unsuccessful. After several attempts, this procedure was abandoned as an approach to the synthesis of α -keto acids.

Synthesis of 5-Hydroxyindolepyruvic Acid

The first reported syntheses of indolepyruvic acid, which involved the condensation of indole-3-carboxaldehyde with hippuric acid (70) or with rhodanine (71), have been reported by Bentley *et al* (72) to be unsatisfactory. Bentley prepared indolepyruvic acid in 46% crude yield from the condensation of indole-3-carboxaldehyde with hydantoin. This procedure would probably be of little value in preparing 5-hydroxyindolepyruvic acid due to the low reactivity of the precursor aldehyde, 5-benzyloxyindole-3-carboxaldehyde (see discussion on the synthesis of 5-hydroxyindole-3-acrylic acid). Shaw, *et al* (73), prepared indolepyruvic acid in 43% overall yield from DL-tryptophan via its N-chloroacetyl derivative (25) (fig. 6) and 2-methyl-4-(3'-indolal)-5-oxazolone (27).

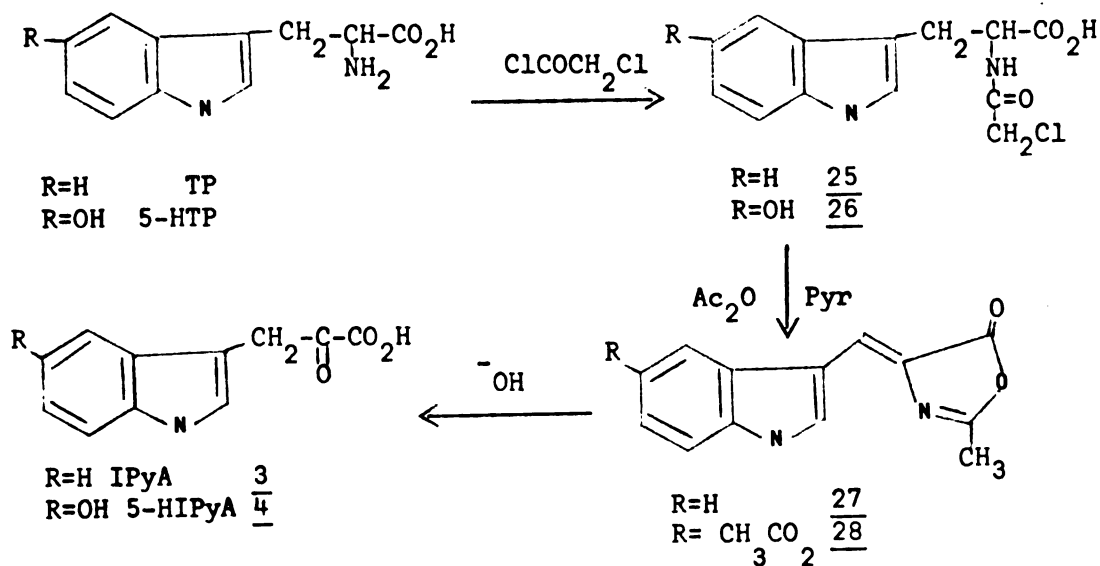


Fig. 6 Synthesis of 5-hydroxyindole-3-pyruvic acid.

1. $\frac{1}{2} \times \frac{1}{2} = \frac{1}{4}$

2. $\frac{1}{2} \times \frac{1}{3} = \frac{1}{6}$

3. $\frac{1}{2} \times \frac{1}{4} = \frac{1}{8}$

4. $\frac{1}{2} \times \frac{1}{5} = \frac{1}{10}$

5. $\frac{1}{2} \times \frac{1}{6} = \frac{1}{12}$

6. $\frac{1}{2} \times \frac{1}{7} = \frac{1}{14}$

7. $\frac{1}{2} \times \frac{1}{8} = \frac{1}{16}$

8. $\frac{1}{2} \times \frac{1}{9} = \frac{1}{18}$

9. $\frac{1}{2} \times \frac{1}{10} = \frac{1}{20}$

10. $\frac{1}{2} \times \frac{1}{11} = \frac{1}{22}$

11. $\frac{1}{2} \times \frac{1}{12} = \frac{1}{24}$

12. $\frac{1}{2} \times \frac{1}{13} = \frac{1}{26}$

13. $\frac{1}{2} \times \frac{1}{14} = \frac{1}{28}$

14. $\frac{1}{2} \times \frac{1}{15} = \frac{1}{30}$

15. $\frac{1}{2} \times \frac{1}{16} = \frac{1}{32}$

16. $\frac{1}{2} \times \frac{1}{17} = \frac{1}{34}$

17. $\frac{1}{2} \times \frac{1}{18} = \frac{1}{36}$

18. $\frac{1}{2} \times \frac{1}{19} = \frac{1}{38}$

19. $\frac{1}{2} \times \frac{1}{20} = \frac{1}{40}$

20. $\frac{1}{2} \times \frac{1}{21} = \frac{1}{42}$

21. $\frac{1}{2} \times \frac{1}{22} = \frac{1}{44}$

22. $\frac{1}{2} \times \frac{1}{23} = \frac{1}{46}$

23. $\frac{1}{2} \times \frac{1}{24} = \frac{1}{48}$

The preparation of 25 by treating DL-tryptophan with chloroacetyl chloride in basic solution is well known (74). The Bergmann rearrangement of 25 with pyridine and acetic anhydride provided the oxazolone (27). Indolepyruvic acid (3) was prepared by Shaw in 67% yield from the oxazolone by alkaline hydrolysis and isolated as the acetic acid solvate.

Using this procedure as a model, 26 was prepared by me by treating DL-5-hydroxytryptophan with chloroacetyl chloride in basic solution. The pH was held at 10.0-11.0 by concurrent addition of NaOH. The N-chloroacetyl compound (26) was not successfully isolated and the oxazolone (28) was prepared directly from crude 26. Recrystallization of crude 28 from benzene gave a bright yellow powder which showed upon mass spectral analysis (electron impact) a mixture of 28 and the 1'-acetyl oxazolone. Shaw also reported the presence of 1'-acetyl oxazolone when the Bergmann rearrangement was allowed to continue for more than a few hours. The mass spectrum also confirmed the absence of any chlorine in the molecule. Sublimation and an additional recrystallization failed to separate the oxazolones so hydrolysis was attempted on the mixture as basic hydrolysis would also cleave the 5'-acetoxy and 1'-acetyl groups. Hydrolysis in 2N NaOH for 6 hrs under N₂ atmosphere yielded a dark powder which, when recrystallized from acetone/benzene with charcoal treatment, gave a brown powder with a wide melting range. The mass spectrum (electron impact) of this material showed a major peak at m/e 191 which is the parent peak for 5-hydroxyindoleacetic acid. Decarbonylation of α -keto acids at mass spectrometer temperatures and energy is known and this would account for the mass 191. After derivatization to the methyl ester and

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2. The second part outlines the various methods and tools used to collect and analyze data. This includes the use of surveys, interviews, and focus groups to gather qualitative information, as well as the application of statistical software for quantitative analysis.

3. The third part details the process of identifying and measuring key performance indicators (KPIs). It explains how these indicators are selected based on the organization's strategic goals and how they are used to monitor progress and performance over time.

4. The fourth part describes the process of reporting and communicating the results of the analysis. It highlights the importance of presenting the data in a clear and concise manner, using visual aids such as charts and graphs to enhance understanding.

5. The final part of the document discusses the implications of the findings and the steps that should be taken to address any identified issues or areas for improvement. It stresses the need for ongoing monitoring and evaluation to ensure that the organization remains on track with its objectives.

methyl ether, the chemical ionization mass spectrum (isobutane) (Table I) showed the major peak at m/e 220 and a minor peak at m/e 248. The m/e 248 could be MH^+ for the derivatized 5-hydroxyindolepyruvic acid and m/e 220 could be MH^+ for derivatized 5-HIAA.

Thin layer chromatography in $CHCl_3/EtOH/HOAc/H_2O$ showed at least three distinct spots at R_f 0, 0.17 and 0.37, all reactive with van Urk's reagent. The decarbonylated product (m_w 219), 5-hydroxyindoleacetic acid, has an R_f value of 0.48, lending support to the theory of decarbonylation in the mass spectrometer. The instability of 5-HIPyA to light and air and the presence of several spots on chromatograms is not surprising. Indolepyruvic acid is unstable in air and light and decomposes extensively in water on paper chromatograms where a fast reaction would be expected with a thin film exposed to air (72,73). In addition, a 5-hydroxyindole would be additionally sensitive to basic conditions. Attempts to further purify the product through recrystallization as the acetic acid or dioxane solvates were fruitless. Isolation and characterization of 5-hydroxyindolepyruvic acid still remains to be accomplished.

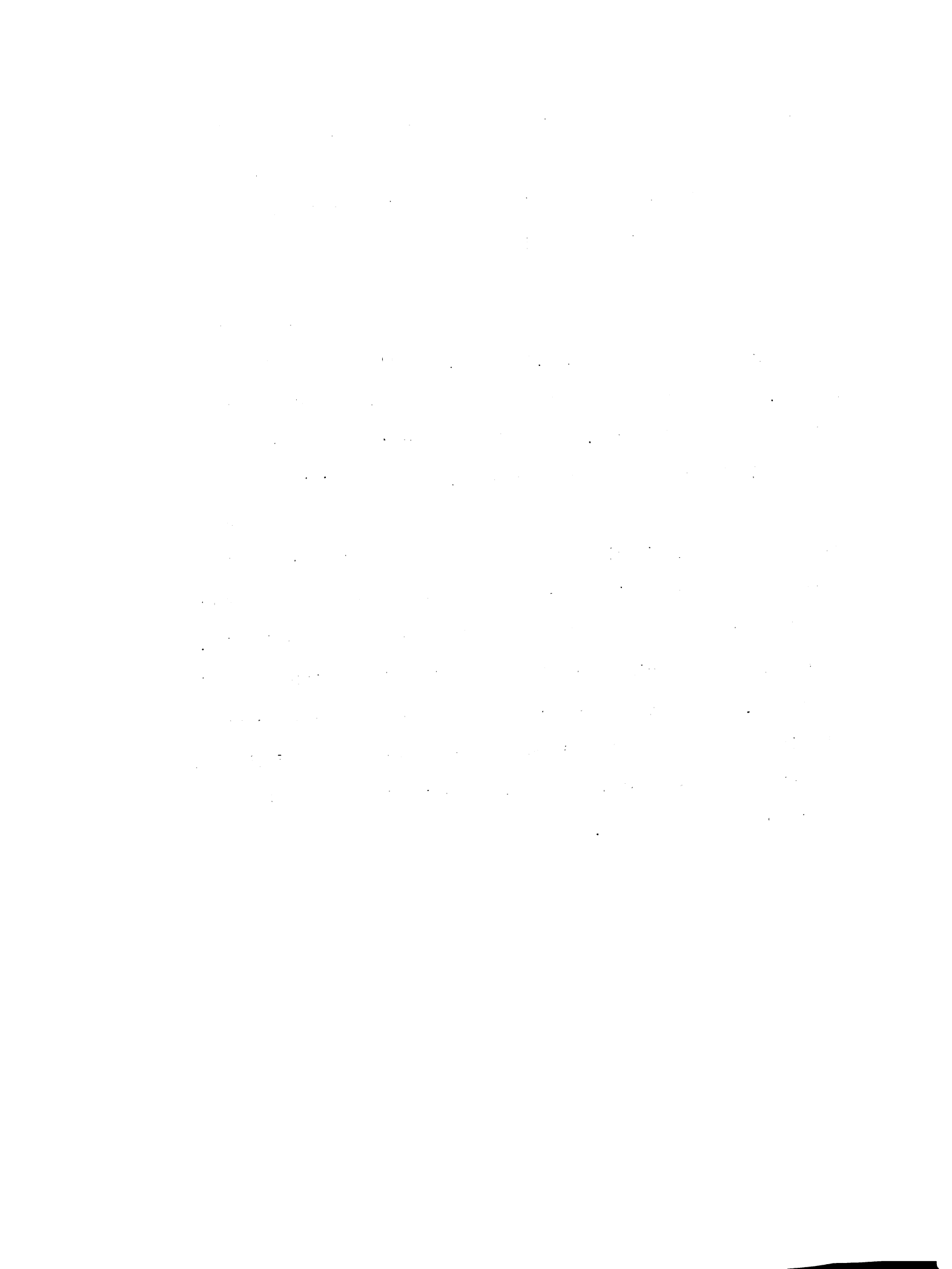


Table 1

Chemical ionization mass spectrum (isobutane) of compound isolated from the reaction mixture of the attempt to synthesize and isolate 5-hydroxyindolepyruvic acid. (treated with diazomethane)

<u>mass</u>	<u>possible compound</u>	<u>relative intensity</u>
304	M + 57 5-HIPyA	5
290	M + 43 5-HIPyA	5.5
286	M + 39 5-HIPyA	2.5
276	M + 57 5-HIAA	8
262	M + 43 5-HIAA	20
258	M + 39 5-HIAA	7
248	MH ⁺ 5-HIPyA methylester-methylether	16
220	MH ⁺ 5-HIAA methylester-methylether	200
188	MH ⁺ -HCO ₂ CH ₃ 5-HIPyA	13
160	MH ⁺ -HCO ₂ CH ₃ 5-HIAA	19

Enzymatic Preparation of Indolepyruvic Acid and 5-Hydroxyindolepyruvic Acid

The enzyme L-amino acid oxidase from the venom of *Crotalus adamanteus* (rattlesnake) can be employed for the preparation of α -keto acids from α -amino acids (75,76). Millard and Gal used this enzyme to prepare 5-hydroxyindolepyruvic acid for use as a standard. (64). They made no attempt to isolate the free acid and reported no spectral data to support its presence. Characterization was based on thin layer chromatography of the 2,4-dinitrophenylhydrazone derivative. I attempted to prepare both indolepyruvic acid and its 5-hydroxy analog by this method.

Methods and Procedure

To a solution of 2.0 mmol amino acid in 40 ml 0.2M tris buffer, pH 7.2, was added 40 mg L-amino acid oxidase (crude *Crotalus adamenteus* venom, Cal Biochem) and 2.5 mg catalase (grade B, Cal Biochem). The total volume was adjusted to 60 ml with buffer which gave a final amino acid concentration of 0.33M. The mixture was allowed to incubate at 37° in the air for 6 hr. The incubation was halted by addition of 1.0 ml conc. HCl, and extracted with ethyl acetate. The ethyl acetate fractions were combined, dried over Na₂SO₄, treated with charcoal and concentrated in vacuo to a brown oil. A small sample of the brown oil was treated with diazomethane and submitted to chemical ionization mass spectral analysis. Thin layer chromatography of the oil was conducted on Eastman 6060 Silica gel and developed in chloroform ethyl acetate formic acid (35:55:10).

Results and Discussion

Table 2 shows the results of an incubation of L-tryptophan with L-amino acid oxidase. Comparative R_f values for standard indolepyruvic acid (Sigma Chemical Co.) and the ethyl acetate extract of the incubate are very close.

The results of an incubation of DL-5-hydroxytryptophan are shown in table 3. The ethyl acetate extract was co-chromatographed with a sample of the brown powder which was the product of the attempt to synthesize and isolate 5-hydroxyindolepyruvic acid. Both samples produced similar values in two solvent systems (table 4). Both

1. The first part of the document discusses the importance of maintaining accurate records of all transactions and activities. It emphasizes that proper record-keeping is essential for transparency and accountability, particularly in financial reporting and compliance with regulatory requirements. The text notes that without reliable records, organizations may face significant challenges in identifying discrepancies, resolving disputes, and demonstrating adherence to applicable laws and standards.

2. The second section addresses the role of internal controls in preventing fraud and errors. It highlights that a robust system of internal controls, including segregation of duties, authorization procedures, and regular audits, is critical for ensuring the integrity of an organization's financial statements. The document stresses that these controls should be designed to detect and prevent potential risks before they result in material misstatements or losses.

3. The third part of the document focuses on the importance of communication and collaboration between different departments and stakeholders. It argues that effective communication is necessary for the successful implementation of any initiative, as it ensures that all parties involved are aligned with the organization's goals and objectives. The text suggests that regular meetings, clear reporting lines, and open channels of communication can help to foster a culture of transparency and mutual respect.

4. The final section discusses the need for continuous improvement and adaptation to changing circumstances. It notes that organizations must regularly evaluate their processes and procedures to identify areas for improvement and make necessary adjustments. This involves staying up-to-date on industry trends, technological advancements, and regulatory changes, and being willing to embrace innovation and change to remain competitive and resilient in a dynamic market environment.

samples also produced chemical ionization mass spectra (after diazomethane treatment) which contained a large peak at m/e 220. It seems inconceivable that 5-hydroxyindolepyruvic acid would decarboxylate to 5-HIAA (MH^+220 methyl ester-methyl ether) under the synthetic reaction conditions and under the incubation conditions. Yet, decarboxylation in the mass spectrometer under chemical ionization conditions is very unlikely. In addition, 5-HIAA co-chromatographs with these two samples in both solvent systems and the uv spectra are similar (figs. 7 a,b,c,d). In addition, high resolution mass spectral analysis (C.I.M.S.) of the eluted spot at R_f 0.87 showed (table 4) m/e 220.0974, which is 1.7 $m\mu$ difference from the molecular formula $C_{12}H_{14}NO_3$, the molecular formula for 5-hydroxyindoleacetic acid methyl ester, methyl ether. Thus, despite the absence of any rational, I must conclude that 5-HIAA was formed in the synthetic reaction and the enzymatic incubation.

I was unable to prepare either the 2,4-dinitrophenylhydrazone or the semicarbazone derivatives from the ethyl acetate fraction of the incubation. The spot of R_f 0.68 from the incubate (table 3) was eluted and the chemical ionization mass spectrum (after treatment with diazomethane) showed peaks at m/e 248 and 234 which could represent the methyl ester - methyl ether and the methyl ester only respectively. This is consistent with the C.I.M.S. of authentic IPyA treated with diazomethane.

Table 2

Thin layer chromatography of L-tryptophan incubated with *Crotalus adamanteus* venom.

<u>Compound</u>	<u>Rf</u>	<u>Color with van Urk's reagent</u>
L-tryptophan	0.20	blue
Authentic indolepyruvic acid	0.75	yellow
incubate	0.25	blue
	0.72	yellow
	0.83	purple
ethyl acetate extract	0.74	yellow
	0.84	purple

Solvent: $\text{CHCl}_3/\text{EtOAc}/\text{HCOOH}$, 35:55:10 (by volume)

Table 3

Thin layer chromatography of DL-5-hydroxytryptophan incubated with *Crotalus adamenteus* venom.

<u>Compound</u>	<u>R_f</u>	<u>Color with van Urk's reagent</u>
DL-5-HTP	0.15	blue
incubate	0.13	blue
	0.42	yellow
	0.68	blue
ethyl acetate extract	0.51	yellow
	0.68	blue
product from synthetic reaction (5HIPyA synthesis)	0 - 0.43	none
	0.68	blue
authentic 5-HIAA	0.68	blue

Solvent: CHCl₃/EtOAc/HCOOH, 35:55:10 (by volume)

Table 4

Thin layer chromatography of extracts from attempted enzymatic and synthetic preparations of 5-hydroxyindolepyruvic acid.

<u>Compound</u>	<u>Rf</u>	<u>Color with van Urk's reagent</u>
ethyl acetate extract from 5-HTP/venom incubate	0-0.63	reddish-purple
	0.74	purple
	0.88	blue
product from synthetic reaction	0.22	pink
	0.28-0.51	purple
	0.60	purple
	0.69	reddish-purple
	0.87	blue
	0.88	blue
authentic 5-HIAA	0.88	blue

Solvent: acetone/CHCl₃/HOAc/H₂O, 40:40:20:5 (by volume)

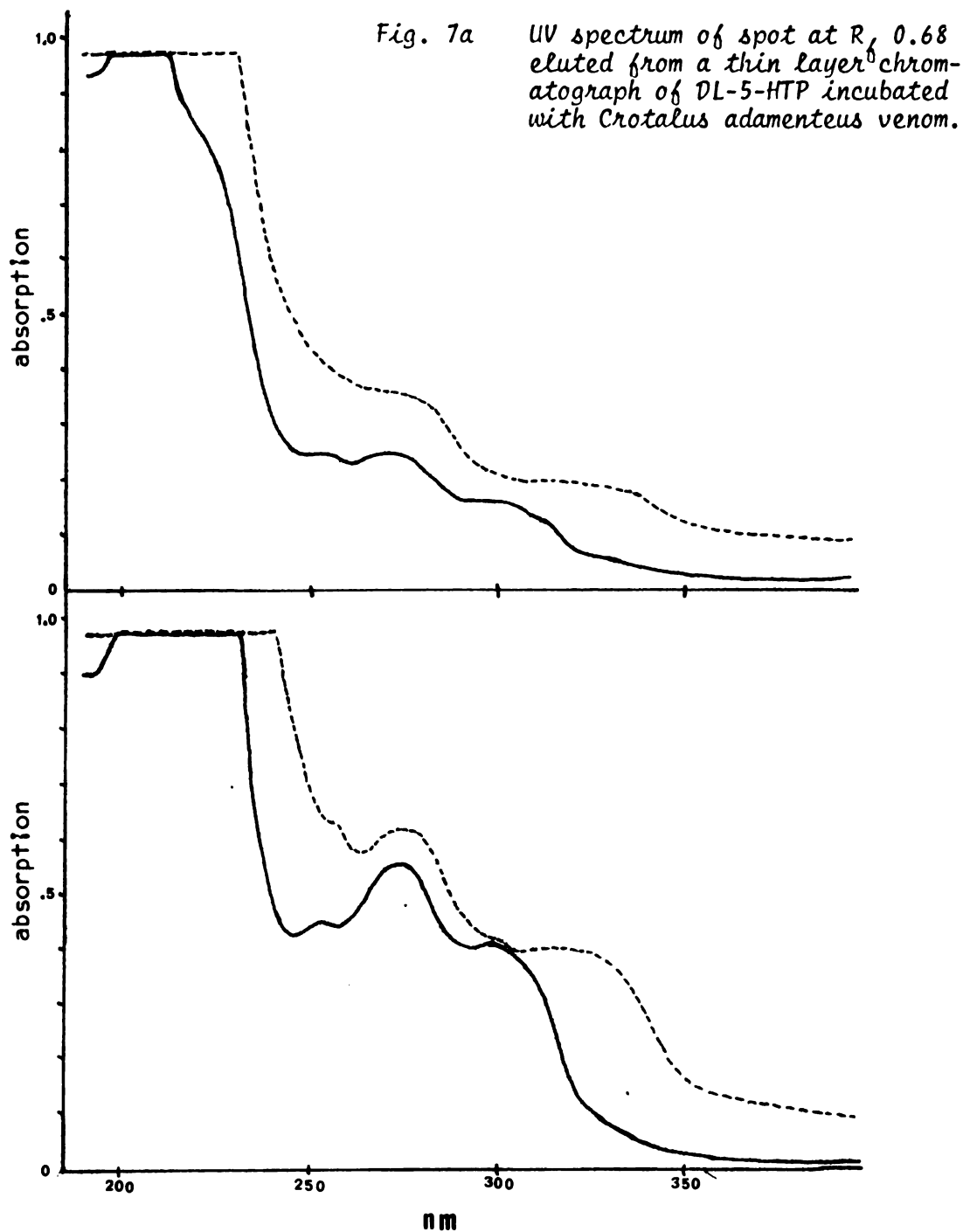


Fig. 7b UV spectrum of spot at R_f 0.88 eluted from a thin layer chromatography of an extract of an incubation of DL-5-HTP with *Crotalus adamanteus* venom.

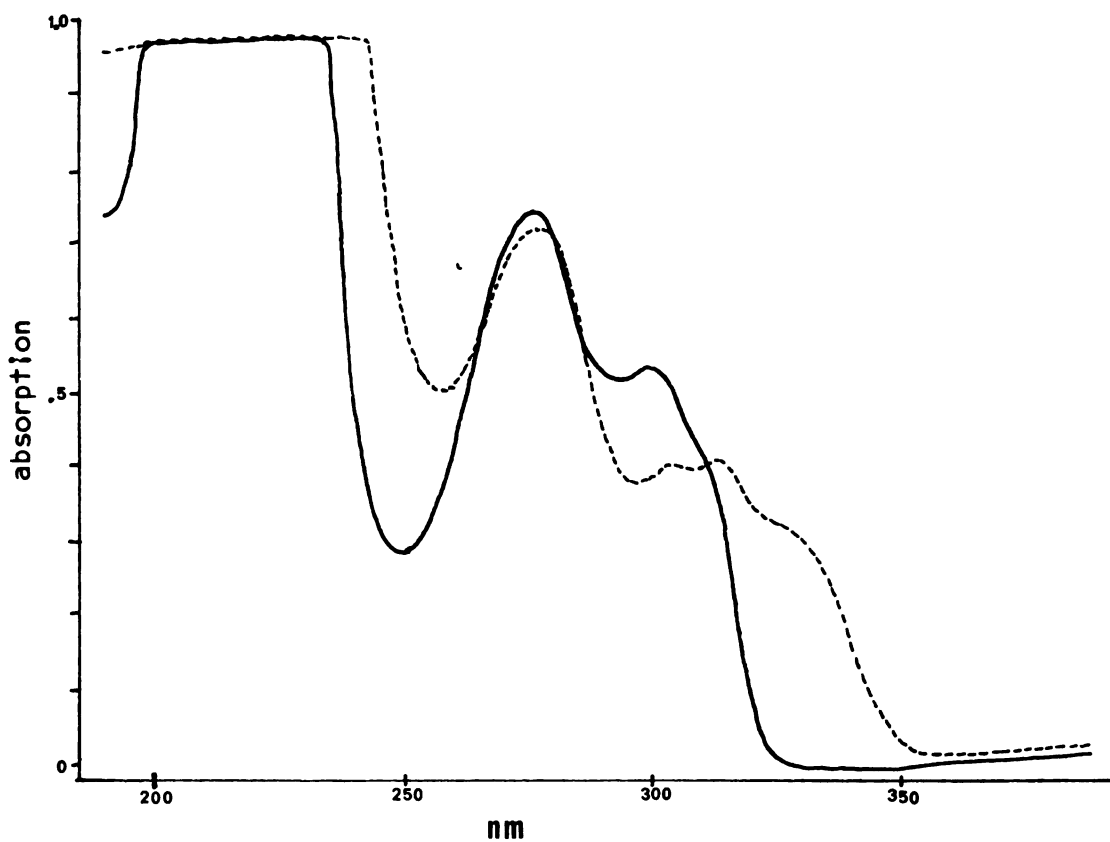
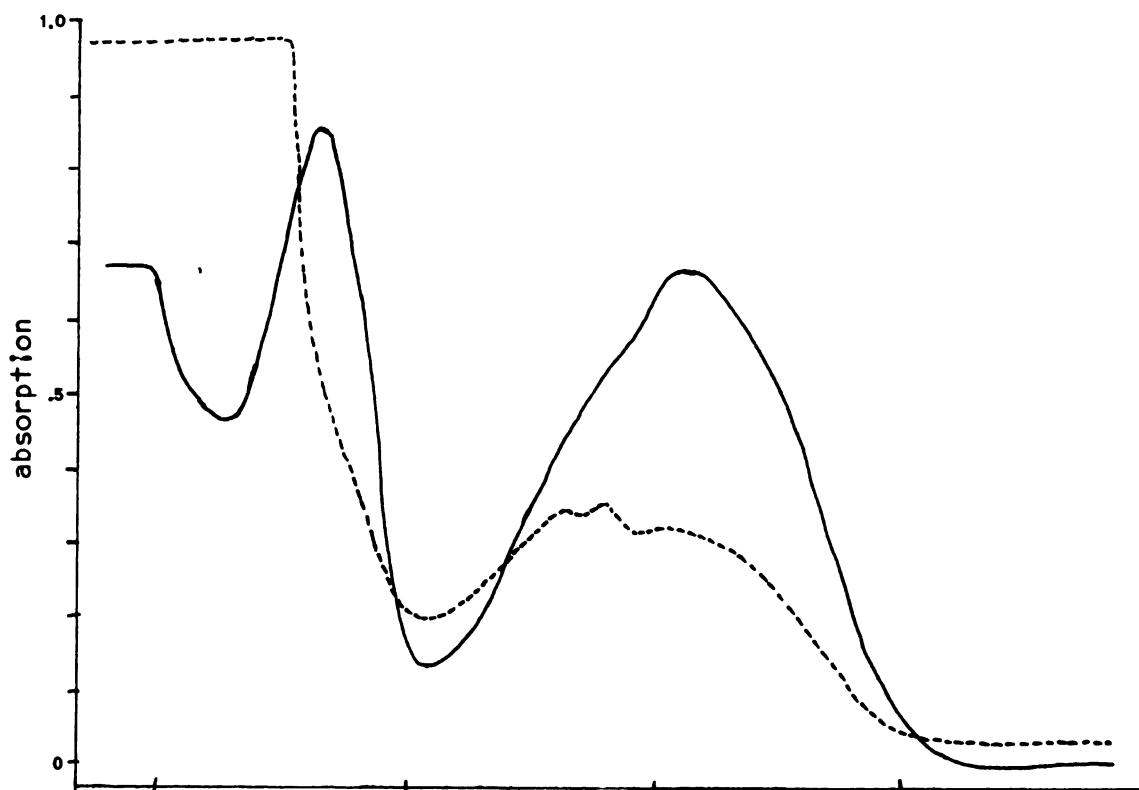


Fig. 7c TOP UV spectrum of indole-3-pyruvic acid.
 — 2% in abs. EtOH
 - - - plus 1 drop 1.0 M NaOH

Fig. 7d Bottom UV spectrum of 5-HIAA.
 — 2% in abs. EtOH
 - - - plus 1 drop 1.0 M NaOH

5-Hydroxyindoleacrylic Acid

Introduction

Preliminary work by Williams and Trager has established the presence of indole-3-acrylic acid in the urine of carcinoid patients. Armstrong *et al* (77) in a survey of the excretion of indole acids and their conjugates in human urine, reported the presence of indoleacrylic acid, but gave no quantitative values or frequency of its occurrence. The free acid and its glycine conjugate, indolylacryloylglycine (IAcryGly), have been found in normal children and adults, those with a chromosomal abnormality (78), patients with Hartnups disease (79) and in a group of East Africans who have a high intake of bananas in their diet. Mandell and Rubin (80) reported IAcryGly excretion as a normal metabolite in man in values ranging from 1.6 to 6.4 mg/24 hr.

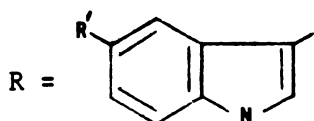
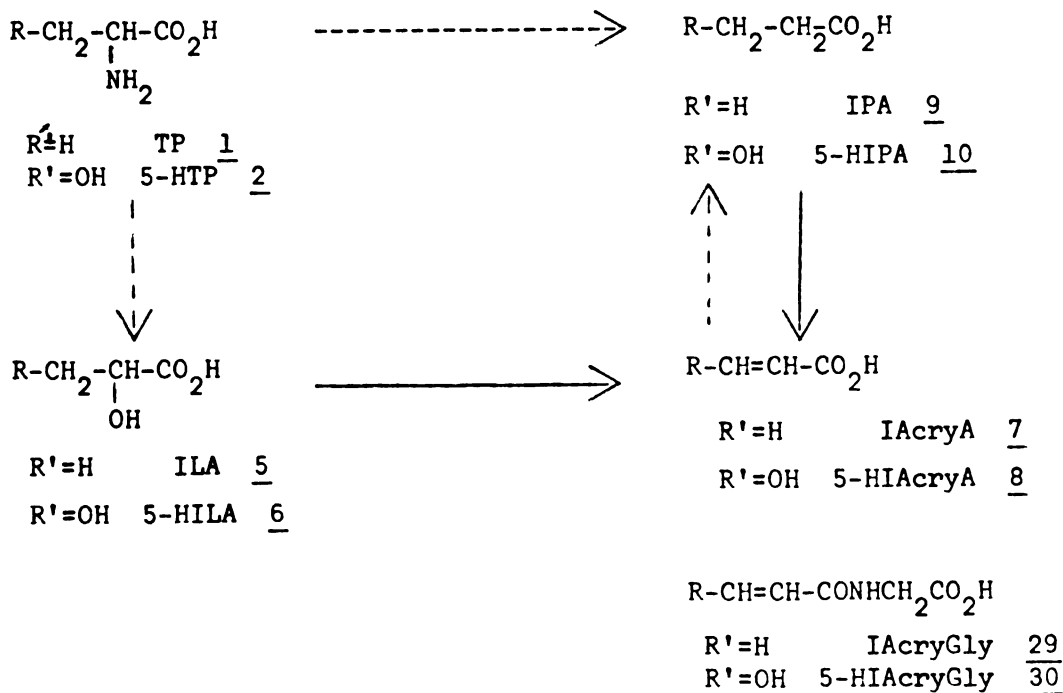


Fig. 8

Hypothetical biosynthetic formation of indole-3-acrylic acid.



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2. The second part of the document focuses on the implementation of robust risk management strategies. It outlines various risk assessment techniques and provides guidance on how to identify, measure, and mitigate potential risks. The text stresses the need for a proactive approach to risk management to protect the organization's assets and reputation.

3. The third part of the document addresses the importance of effective communication and reporting. It discusses the need for clear and concise communication channels and the role of regular reporting in keeping stakeholders informed. This section also touches upon the importance of maintaining accurate financial statements and providing timely updates to management and investors.

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10. The tenth part of the document discusses the importance of maintaining accurate records of all transactions and activities. It emphasizes that proper record-keeping is essential for ensuring transparency and accountability in financial operations. This section also highlights the role of internal controls in preventing fraud and errors.

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12. The twelfth part of the document addresses the importance of effective communication and reporting. It discusses the need for clear and concise communication channels and the role of regular reporting in keeping stakeholders informed. This section also touches upon the importance of maintaining accurate financial statements and providing timely updates to management and investors.

The mechanism of formation of indoleacrylic acid remains to be fully investigated. Jepson et al (81) suggest that tryptophan is first metabolized to indolepropionic acid by intestinal flora and then to indoleacrylic acid in the liver or kidney. Another pathway may be via indolelactic acid. Urinary excretion of indoleacrylic acid has been reported in six children with phenylketonuria (82) and these subjects also have indolelactic acid present in their urine (83). It is therefore established that indoleacrylic acid and its conjugate are urinary metabolites in normals and in patients with certain diseases. It seemed logical that if this indolic acid were present in the urine that it may be possible that its hydroxylated "counterpart", 5-hydroxyindoleacrylic acid (8) may also be present, particularly in carcinoid patients where there are large amounts of 5-hydroxylated indole metabolites excreted. In order to establish unambiguously the presence of this metabolite it was necessary to synthesize an authentic standard.

Synthesis

β -3-Indoleacrylic acid (7) has been synthesized by Shaw et al (73) in 50% yield employing the Doebner modification of the Knoevenagel reaction. (For a review see 84). The Doebner reaction involves the condensation of malonic acid with 3-indolecarboxaldehyde under basic conditions, (pyridine with a catalytic amount of piperidine), with subsequent decarboxylation and dehydration to give the α , β -unsaturated acid (fig. 9). The trans olefin is always formed. Rate studies by Corey (85), Patai (86) and Gensler (87) on the Doebner reaction show that dehydration precedes decarboxylation.

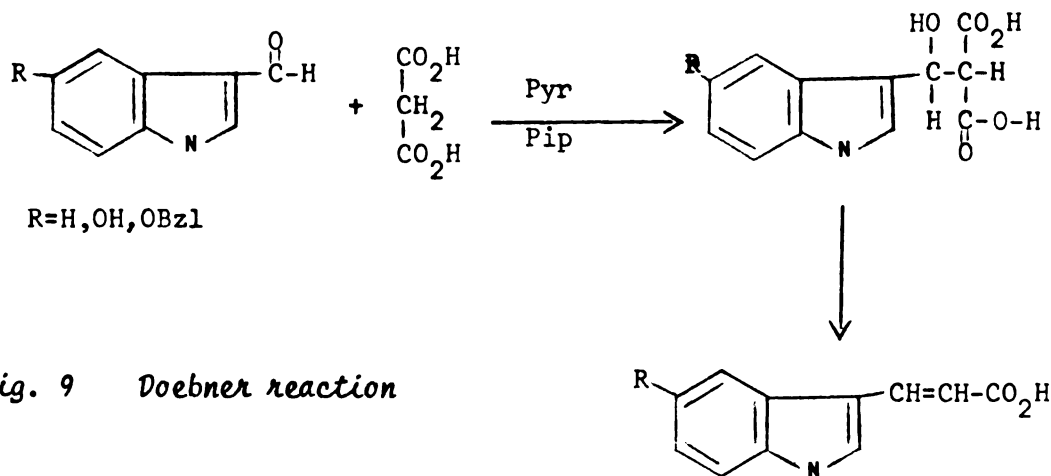
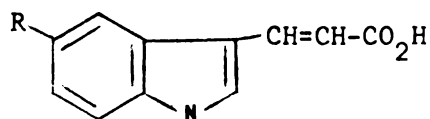


Fig. 9 Doebner reaction



R=H 7

R=OH 8

A major determinant of reactivity in this reaction is the type of carbonyl involved. These aldol-like condensations are successful only with aldehydes that have sufficient carbonyl character, that is, the carbonyl carbon is sufficiently electrophilic. Para substituted benzaldehydes will react faster or slower depending upon whether the para substituent is electron withdrawing or electron donating thus increasing or decreasing the electrophilicity of the aldehydic carbon.

the fact that the system is not a simple linear system, but a nonlinear system. The nonlinear system is a system in which the output is not directly proportional to the input. In other words, the system does not follow the principle of superposition. This means that the system's response to a combination of inputs is not the same as the sum of its responses to each input individually. This is a key characteristic of nonlinear systems, and it is what makes them so difficult to analyze and design. In the context of the control system, the nonlinear system is the plant, and the linear system is the controller. The controller is designed to stabilize the plant, but it must take into account the nonlinear nature of the plant. This is why the controller is often designed using techniques that are specifically tailored to nonlinear systems, such as Lyapunov's method or backstepping.

The system is a nonlinear system, and the controller is a linear system. The system is nonlinear because it contains a nonlinear element, which is the plant. The controller is linear because it is a transfer function, and transfer functions are linear. The system is a closed-loop system, and the controller is a feedback controller. The system is a control system, and the controller is a control law. The system is a dynamic system, and the controller is a static system. The system is a continuous-time system, and the controller is a discrete-time system. The system is a single-input, single-output system, and the controller is a single-input, single-output system. The system is a linear system, and the controller is a linear system. The system is a nonlinear system, and the controller is a nonlinear system. The system is a linear system, and the controller is a nonlinear system. The system is a nonlinear system, and the controller is a linear system. The system is a linear system, and the controller is a nonlinear system.

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p-Methoxybenzaldehyde reacts very slowly or not at all while the reaction rate is much increased for p-nitrobenzaldehyde.

Indole is a very strong electron donor and is a good nucleophile in its own right, reacting with electrophiles at the three position of the indole ring. It is thus not surprising that indole-3-carboxaldehyde reacts in the Doebner reaction with more difficulty than benzaldehyde. Furthermore, the addition of another electron donating group to the indole ring such as hydroxy or benzyloxy may serve to further increase the electron density of the indole ring and thus further reduce the electrophilicity of the aldehydic carbon.

I have had no success in synthesizing 5-hydroxyindoleacrylic acid via the Doebner reaction. All indications point to the decreased carbonyl reactivity of the starting material, 5-benzyloxyindole-3-carboxaldehyde, as the reason behind the non-reactivity. The Doebner reaction was used however, to prepare cinnamic acid from benzaldehyde in 64% yield and N-acetyl-indole-3-acrylic acid in 50% yield. In all, six attempts using 5-benzyloxyindole-3-carboxaldehyde in the Doebner reaction and one attempt employing acetic anhydride and sodium acetate (Perkin reaction) ended in failure. In all the reactions, unreacted starting material was recovered in yields of 50-80% with no indication that any of the desired product was formed. Infrared and ultraviolet spectra of 5-benzyloxyindole-3-carboxaldehyde support the supposition that the aldehydic carbon is of such decreased carbonyl character so as to be unreactive toward the type of nucleophile generated from malonic acid. (Diethylmalonate was equally unreactive.) Figures 10 and 11a,b show the i.r. and u.v. spectra. The major carbonyl band is located at

...and the fact that the system is not yet fully operational, the Commission has decided to suspend the payment of the first instalment of the aid.

The Commission has also decided to suspend the payment of the second instalment of the aid, pending the submission of the final report on the progress of the implementation of the aid. The Commission will also suspend the payment of the third instalment of the aid, pending the submission of the final report on the progress of the implementation of the aid.

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a very low frequency - 1629 cm^{-1} - which seems to indicate a lower degree of carbonyl character than most aldehydes. The frequency is more like that of an amide carbonyl than an aldehyde carbonyl. The u.v. spectrum shows a bathochromatic shift when aqueous base is added (fig. 11a). No shift occurs when aqueous acid is added (fig. 11b). Again, this seems to suggest a further decrease in carbonyl character of the aldehyde when in solution under basic conditions. A sample of 5-benzyloxyindole-3-carboxaldehyde was sent to Mr. Tom Tenforde at the University of California, Berkeley, for more extensive u.v. and i.r. analysis. He reports that the u.v. spectra showed similar λ_{max} 's at comparable wavelengths in EtOH, DMSO and CCl_4 . Addition of alkali to the aldehyde in EtOH and DMSO shifted the peaks to longer wavelengths by ca. 20nm. The i.r. spectrum in nujol showed the major carbonyl band at 1629 cm^{-1} , with several shoulders between 1630 and 1670 cm^{-1} . A 5X expanded scale spectrum in EtOH showed 4 major carbonyl bands at 1630 , 1640 , 1650 and 1657 cm^{-1} . His interpretation is that the spectra are consistent with extensive intermolecular hydrogen bonding. The fact that there appears to be several carbonyl bands indicates the presence of a number of different H-bonded conformations. With regard to the effect of alkali on the u.v. spectra, he suggests that hydroxyl ion could enter into aldehyde H-bonding in a complicated manner. It is possible that H-bonding leads to stacking of the benzyloxy-indolecarboxaldehyde molecules with overlap of π electron clouds. A change in H-bonding conformation in the presence of alkali could then greatly alter the electronic transition frequencies.

1. The first step in the process of identifying a problem is to recognize that a problem exists. This is often done by comparing current performance with a desired state or goal. For example, a manager might notice that sales are declining or that customer satisfaction is low. Once a problem is identified, the next step is to define it more precisely. This involves determining the scope of the problem, its causes, and its effects. A clear definition of the problem is essential for developing an effective solution.

2. The second step is to gather information about the problem. This can be done through various methods, such as interviews, surveys, and data analysis. The goal is to understand the underlying causes of the problem and to identify any constraints or resources that may affect the solution. For example, a manager might interview employees to learn about their experiences with a particular process or analyze sales data to identify trends and patterns.

3. The third step is to generate potential solutions. This is often done through brainstorming or other creative techniques. The goal is to come up with a range of possible solutions that could address the problem. It is important to consider both short-term and long-term solutions, as well as solutions that are feasible and sustainable. For example, a manager might brainstorm ideas for improving customer service, such as offering more personalized service or improving the quality of products.

4. The fourth step is to evaluate the potential solutions. This involves comparing the different solutions against a set of criteria, such as cost, time, and effectiveness. The goal is to identify the most promising solution and to determine whether it is feasible and sustainable. For example, a manager might evaluate different solutions for improving customer service based on their cost, the time it would take to implement them, and their potential to improve customer satisfaction.

5. The fifth and final step is to implement the chosen solution. This involves putting the solution into action and monitoring its progress. It is important to communicate the solution to all relevant parties and to provide them with the resources and support they need to implement it. Additionally, it is important to monitor the solution's performance and to make adjustments as needed. For example, a manager might implement a new customer service process and then monitor customer satisfaction levels to see if the process is having the desired effect.

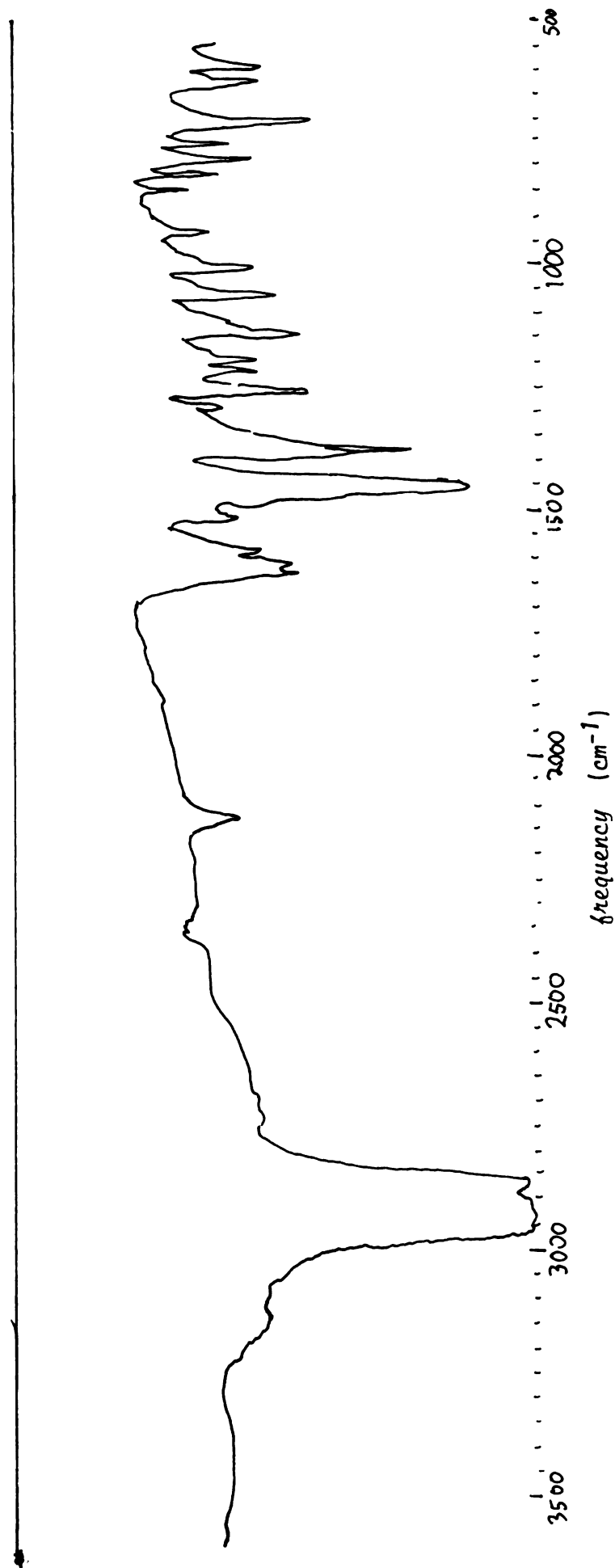


Fig. 10 Infrared spectrum of 5-benzylloxyindole-3-carboxaldehyde.
(crystalline dispersion in Nujol)

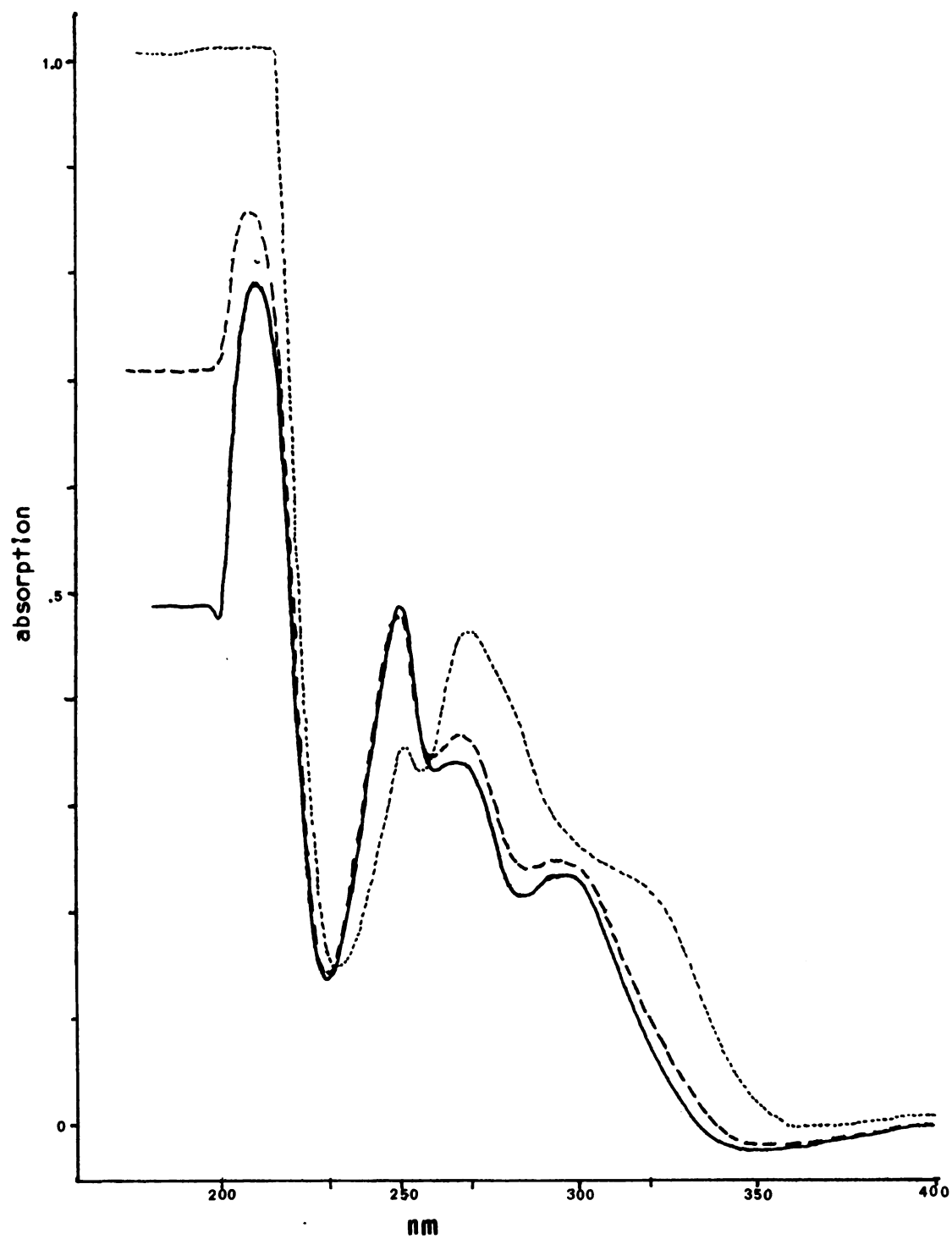


Fig. 11a UV spectrum of 5-benzyloxyindole-3-carboxaldehyde.

— 2% in abs. EtOH
- - - plus 1 drop 0.1 N NaOH
... plus 5 drops 0.1 N NaOH

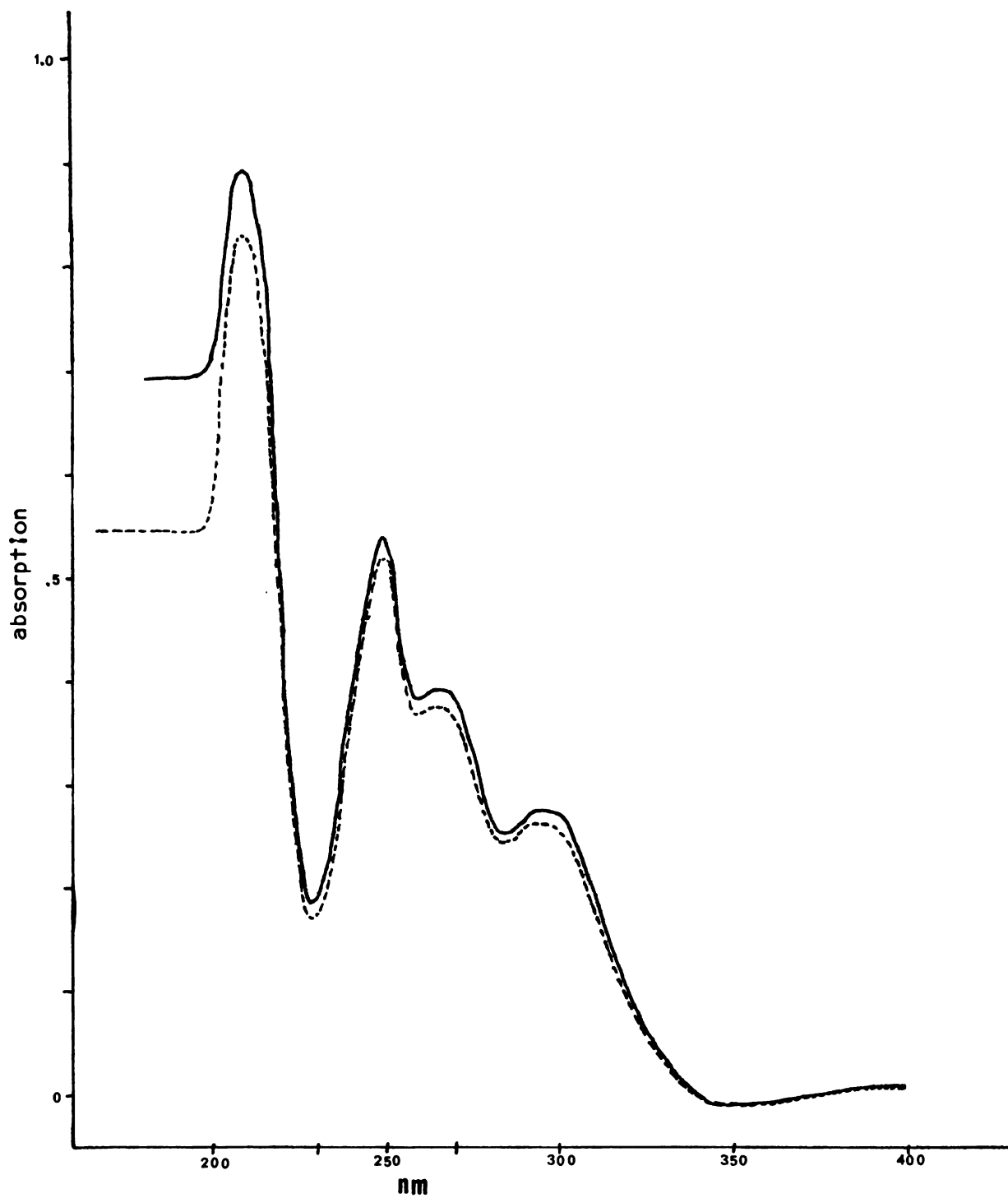


Fig. 11b UV spectrum of 5-benzyloxyindole-3-carboxaldehyde.

— 2% in abs. EtOH
- - - plus 5 drops 0.1 N HCl

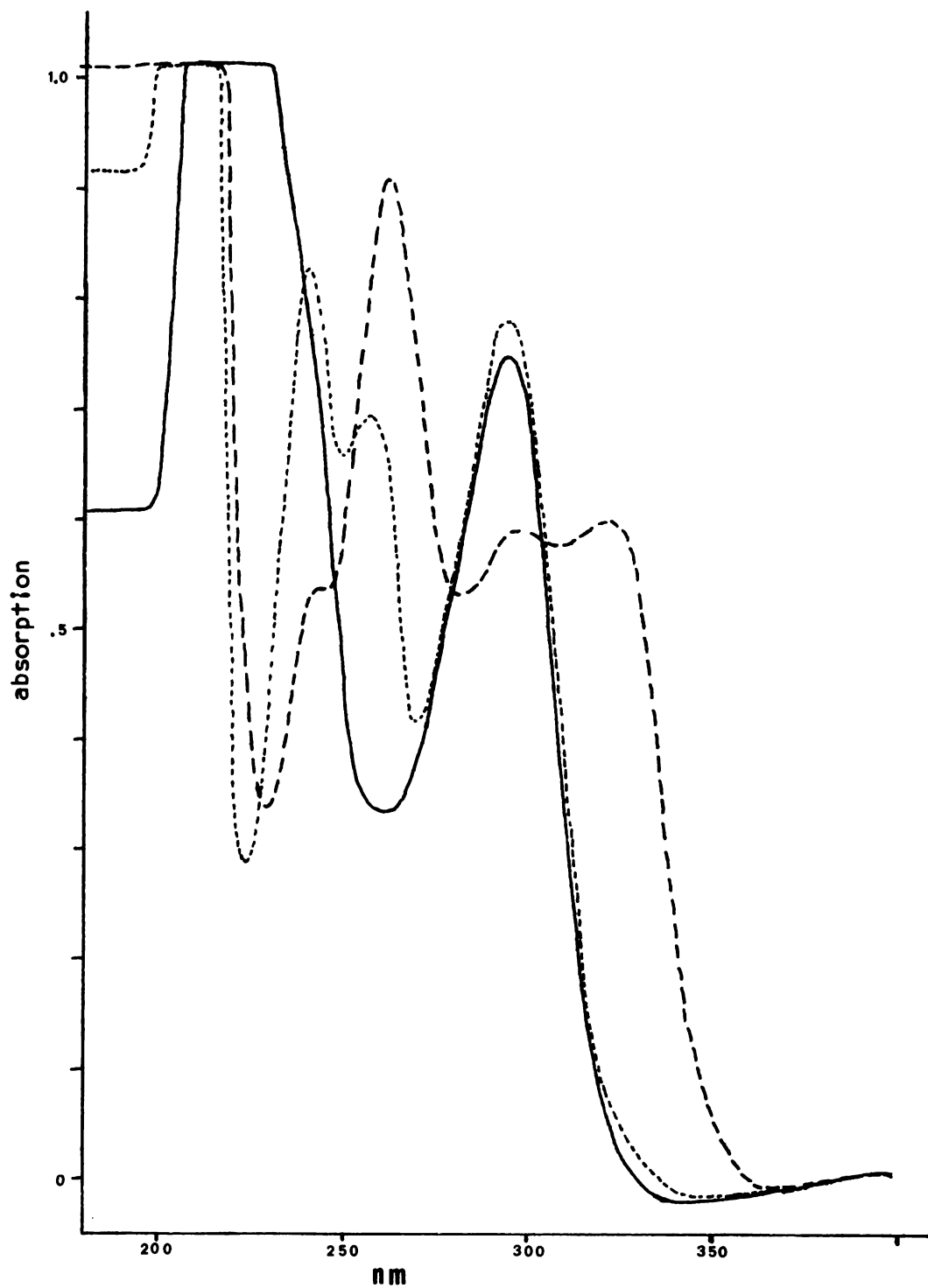


Fig. 12 UV spectrum of *N*-acetylindole-3-carboxaldehyde.

— 1.5% in abs. EtOH
--- plus 1 drop 1.0 N NaOH
.... plus 2 drops 1.0 N HCl

If intermolecular hydrogen bonding and stacking of the molecules does indeed occur, that along with the decreased carbonyl character of the aldehyde moiety may serve to explain this compound being refractory to the Doebner reaction. Stacking of the molecules in solution and intermolecular H-bonding may sterically inhibit attack by any nucleophile. That combined with decreased electrophilicity and the fact that the enolate ion of malonic acid would be a weak nucleophile and in low concentration, would be sufficient to block reaction. It is interesting to note that similar difficulties with such reactions involving 5-benzyloxy or 5-hydroxy substituted benzothiothene derivatives have been reported (88).

Another approach to synthesis of 5-hydroxyindoleacrylic acid is to decrease the electron donating properties of the indole ring. An N-acetyl group on 5-benzyloxyindole-3-carboxaldehyde would serve to decrease the electron density in the indole ring and hence at the aldehyde group. However, cleavage of the N-acetyl compound to give the free indole must be done in strong aqueous base. It is known from previous work done by me that 5-hydroxyindoles are very base sensitive and long periods of reflux in concentrations of aqueous base high enough to cleave an amide would result in massive decomposition. This approach was therefore abandoned.

A third approach to the synthesis of 5-hydroxyindole-3-acrylic acid was to utilize the nucleophilicity of the indole ring in a reaction involving a Michael addition to an alkyne. Shown in figure 13, the addition of indole to propiolic acid could give the indole substituted acrylic acid. This reaction was tried with indole and

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2. The second step in the process is to analyze the problem. This involves gathering information about the problem and its context. This information can be obtained through various methods, such as interviews, surveys, and data analysis. The goal of this step is to understand the underlying causes of the problem and to identify the factors that are contributing to it. This information is then used to develop a plan of action.

3. The third step in the process is to develop a solution. This involves identifying the most effective and efficient way to address the problem. This is often done by brainstorming ideas and evaluating them against various criteria, such as cost, time, and effectiveness. Once a solution has been identified, the next step is to implement it. This involves putting the solution into action and monitoring its progress.

4. The fourth step in the process is to evaluate the results. This involves comparing the actual results of the solution with the desired state or goal. This information is then used to determine whether the solution was effective and to identify any areas for improvement. If the solution was not effective, the process may need to be repeated.

5. The fifth and final step in the process is to document the solution. This involves creating a record of the problem, the analysis, the solution, and the results. This documentation is important for several reasons. First, it provides a clear record of the problem and the solution, which can be used for future reference. Second, it provides a clear record of the process, which can be used to identify any areas for improvement. Finally, it provides a clear record of the results, which can be used to demonstrate the effectiveness of the solution.

propionic acid in dioxane under reflux conditions. No indoleacrylic acid could be detected by thin layer chromatography after 4 days and this approach was abandoned.

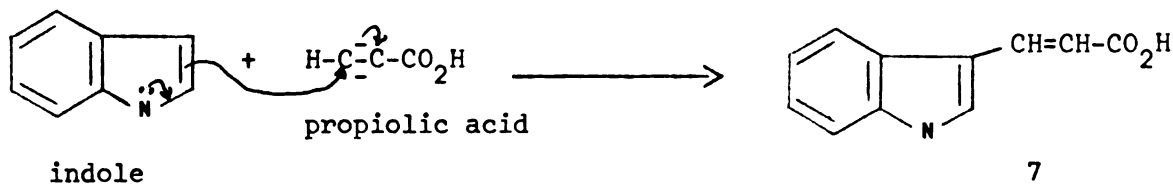


Fig. 13 Possible reaction mechanism for the synthesis of indole-3-acrylic acid.

A fourth approach to the synthesis of 5-hydroxyindole-3-acrylic acid was to use the Grignard salt of the indole. Woodward (89) utilized the Grignard anion of 6-methoxyindole as a nucleophile in the substitution of α -chloroacetonitrile in the synthesis of the tryptamine portion of reserpine. Using this as a model, I employed the Grignard anion of 5-methoxyindole as a nucleophile in the substitution of 3-chloroacrylic acid (fig. 14), following the procedure of Akabori and Saito (90). This is essentially a 1,4-addition of a Grignard to a carbonyl followed by elimination of HCl. Competition between 1,4 and 1,2 addition exists but 1,2-addition is kept to a minimum by steric hinderance of the rather large Grignard reagent. Competition by the acidic proton of the carboxylic acid to give simple acid-base reaction is also probable but evidently is not an overwhelming problem at low temperature. 5-Methoxyindoleacrylic acid was prepared successfully in this manner. Figures 15, 16 and 17 show the uv spectra of 5-methoxyindoleacrylic acid, indoleacrylic acid and the nmr of 5-methoxyindoleacrylic acid respectively.

the \mathbb{R}^n -valued function $f: \mathbb{R}^n \rightarrow \mathbb{R}^n$ is called a *vector field* on \mathbb{R}^n . If $f(x) = (f_1(x), \dots, f_n(x))$, then f is a vector field on \mathbb{R}^n if and only if each f_i is a scalar field on \mathbb{R}^n . For example, the vector field $f(x) = (x, -y)$ on \mathbb{R}^2 is represented by the vector field $f(x, y) = (x, -y)$ in the xy -plane.

Let f be a vector field on \mathbb{R}^n . For each point $x \in \mathbb{R}^n$, the vector $f(x)$ is tangent to the curve $\gamma(t) = x + t f(x)$ at $t = 0$. The curve γ is called a *trajectory* of f starting at x . The set of all trajectories of f is called the *integral curves* of f . The vector field f is said to be *irrotational* if $\text{curl } f = 0$. The vector field f is said to be *solenoidal* if $\text{div } f = 0$.

Let f be a vector field on \mathbb{R}^n . The vector field f is said to be *conservative* if there exists a scalar field ϕ on \mathbb{R}^n such that $f = \nabla \phi$. The scalar field ϕ is called a *potential function* for f . The vector field f is conservative if and only if $\text{curl } f = 0$. The vector field f is solenoidal if and only if $\text{div } f = 0$.

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This reaction was attempted with 5-benzyloxyindole but was not successful. Only starting materials were recovered. Employing a twice molar excess of the Grignard salt of the indole to account for reaction with the acid proton also resulted in failure. Utilizing the methyl ester of 3-chloroacrylic acid was likewise unsuccessful for unknown reasons.

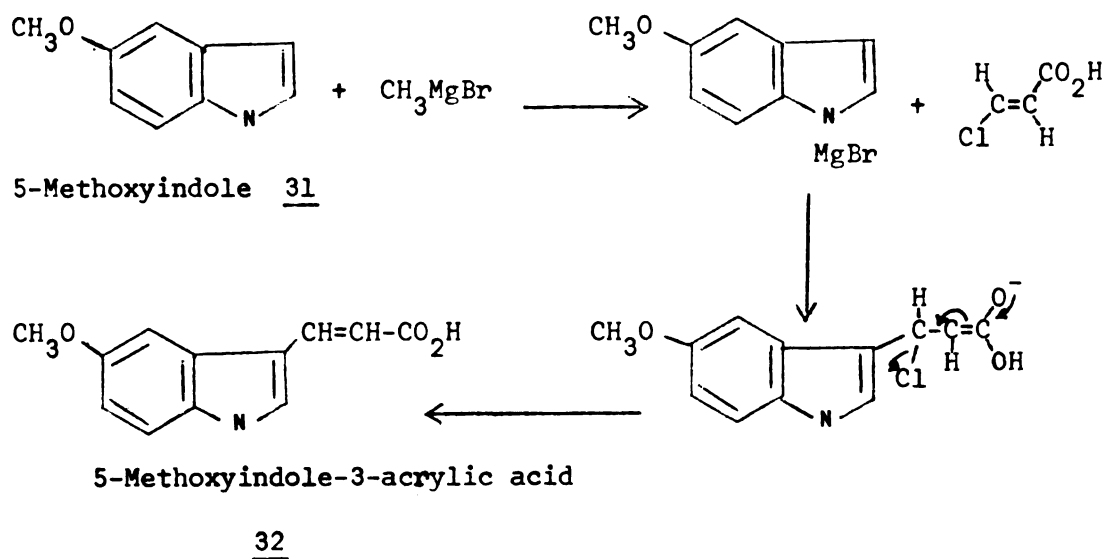


Fig. 14 Synthesis of 5-Methoxyindole-3-acrylic acid.

An attempt was made to utilize the Grignard anion of 5-benzyloxyindole as a nucleophile in the 1,4-addition to propiolic acid or its ester. The reaction with the free acid was fruitless. An attempt employing the ester of propiolic acid was in order. I needed an ester readily cleaved under mild conditions as the free acid was the desired product. The benzyl ester was chosen because it could be cleaved under the same conditions as the benzyl ether. The synthesis of benzyl propiolate was unsuccessful. Following the procedure of Bowie (91), propiolic acid was mixed with benzyl alcohol using H_2SO_4 as a catalyst. Since strong acids such as H_2SO_4 are known to

1. The first step in the process of identifying a problem is to recognize that a problem exists. This is often done by comparing current performance with a desired state or goal. Once a problem is identified, the next step is to define the problem more precisely. This involves determining the scope of the problem, the resources available, and the constraints that may be present. The third step is to analyze the problem to determine its causes. This is often done using tools such as the fishbone diagram or the 5 Whys technique. The fourth step is to generate potential solutions. This is often done by brainstorming or using other creative problem-solving techniques. The fifth step is to evaluate the potential solutions and select the best one. This is often done by comparing the solutions against the criteria established in the first step. The final step is to implement the selected solution and monitor its progress. This is often done by setting up a system of controls and feedback loops.

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catalyze the polymerization of both propiolic acid and benzyl alcohol, it is not surprising that this reaction produced only a large mass of polymeric material.

A second attempt utilizing the well known peptide synthesis reagent, *N,N'*-dicyclohexylcarbodiimide (DCCI), also resulted in failure for unknown reasons. The side product, *N,N'*-dicyclohexylurea was isolated in 60% yield but benzyl propiolate could not be distilled from the reaction mixture at the reported boiling point, 122°/18 mm (91). Various reaction conditions were employed, from room temperature to reflux and a twice molar excess of propiolic acid was used. This approach was fruitless and therefore abandoned.

A fourth attempt to synthesize 5-hydroxyindole-3-acrylic acid via benzyl 5-benzyloxyindole-3-acrylate ended in failure also. It was thought that it would be possible to reduce and dehydrate a β -keto ester 33 to the α,β -unsaturated ester 34 (fig. 18). Synthesis of benzyl 5-benzyloxyindole-3- β -ketopropionate (33) was accomplished by condensing 5-benzyloxyindole with malonyl dichloride and then adding benzyl alcohol. Reduction of the keto group with NaBH_4 was tried in THF/EtOH and in isopropanol. Both reaction conditions produced transesterification products. C.I.M.S. analysis showed the presence of a very small amount of reduced and dehydrated product but it is not known if the dehydrated product is a result of reduction by NaBH_4 or is an artifact of the mass spectral technique. It is conceivable that any reduction product would dehydrate under chemical ionization conditions so readily so as not to show a parent peak. However, since this compound represented less than 1% of the product, this synthetic approach was abandoned.

1. The first step is to identify the main topic of the document.

2. Next, we should look for key points or arguments made by the author.

3. It is important to note any evidence or data used to support the claims.

4. We should also consider the author's perspective and any potential biases.

5. Finally, we need to evaluate the overall quality and credibility of the information.

6. This process helps us understand the document's content and its implications.

7. By following these steps, we can effectively analyze and summarize the text.

8. The goal is to provide a clear and concise overview of the document's main ideas.

9. This approach is useful for both academic and professional purposes.

10. It allows us to quickly grasp the essential information presented in the document.

11. We should always be critical and question the validity of the information.

12. This method ensures that we are well-informed and can make informed decisions.

13. It is a systematic way to approach reading and understanding complex texts.

14. By using this process, we can maximize our understanding and retention of the material.

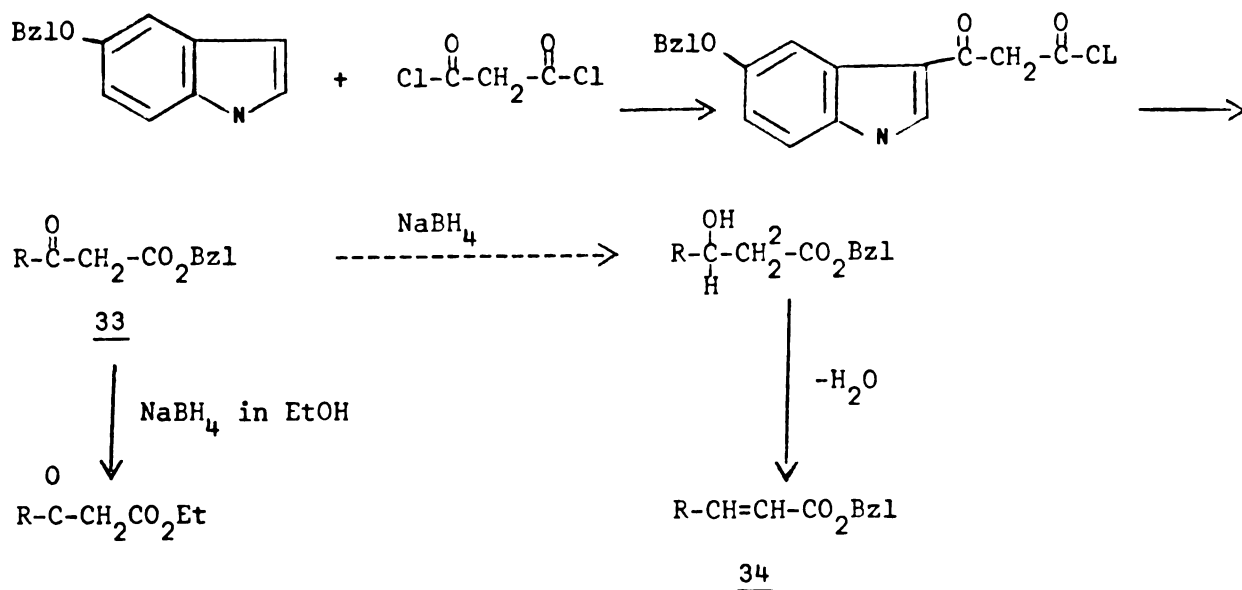


Fig. 18 Possible synthesis of 5-benzyloxyindoleacrylic acid.

The Grignard reaction with propiolic acid was attempted again but this time with 5-methoxyindole instead of 5-benzyloxyindole. Employing a twice molar excess of the Grignard anion of 5-methoxyindole, 5-methoxyindole-3-acrylic acid was prepared in 12% yield. The reaction conditions were the same as for the preparation of the indole substituted acrylic acid when 3-chloroacrylic acid was used. It was therefore apparent that 5-methoxyindole would react, although in very small yields, under these conditions but that for some unknown reason, 5-benzyloxyindole would not. However, since derivatization of all acidic metabolites to the methyl esters and methyl ethers was necessary for the mass spectral assay, it was decided to abandon the attempt to synthesize the free 5-hydroxy compound and be satisfied with the 5-methoxy compound.

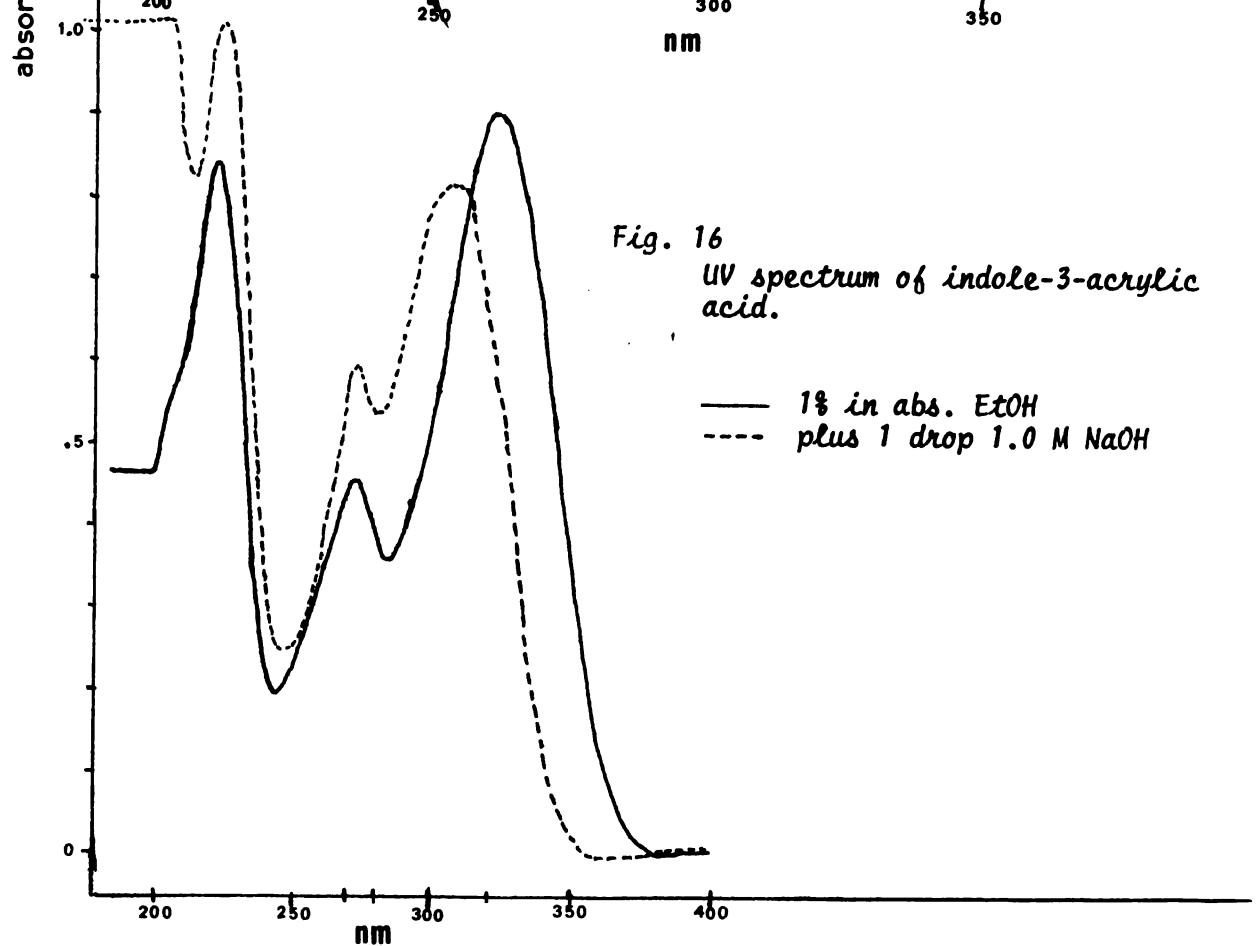
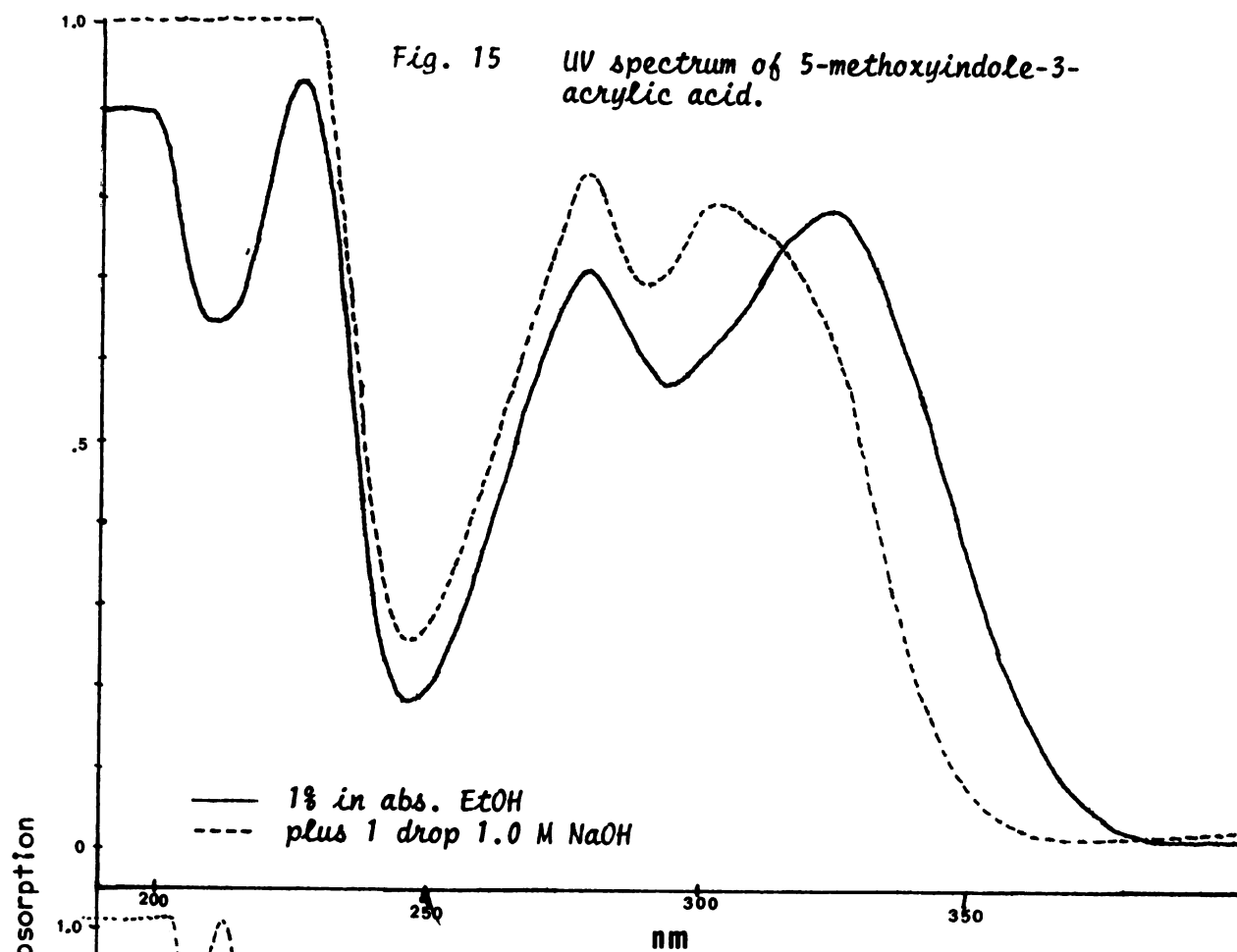
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The first part of the document discusses the importance of maintaining accurate records of all transactions. It emphasizes that every entry should be supported by a valid receipt or invoice. This ensures transparency and allows for easy verification of the data.

In the second section, the author outlines the various methods used to collect and analyze the data. This includes both primary and secondary data collection techniques. The primary data was gathered through direct observation and interviews with key stakeholders. Secondary data was obtained from existing reports and databases.

The analysis phase involved using statistical software to identify trends and correlations within the data. The results show a clear upward trend in the number of transactions over the period studied. There is also a significant correlation between the time of day and the volume of activity.

Finally, the document concludes with a series of recommendations for improving the data collection process. These include implementing more robust data entry protocols, increasing the frequency of data collection, and ensuring that all staff are properly trained in the use of the data management system.



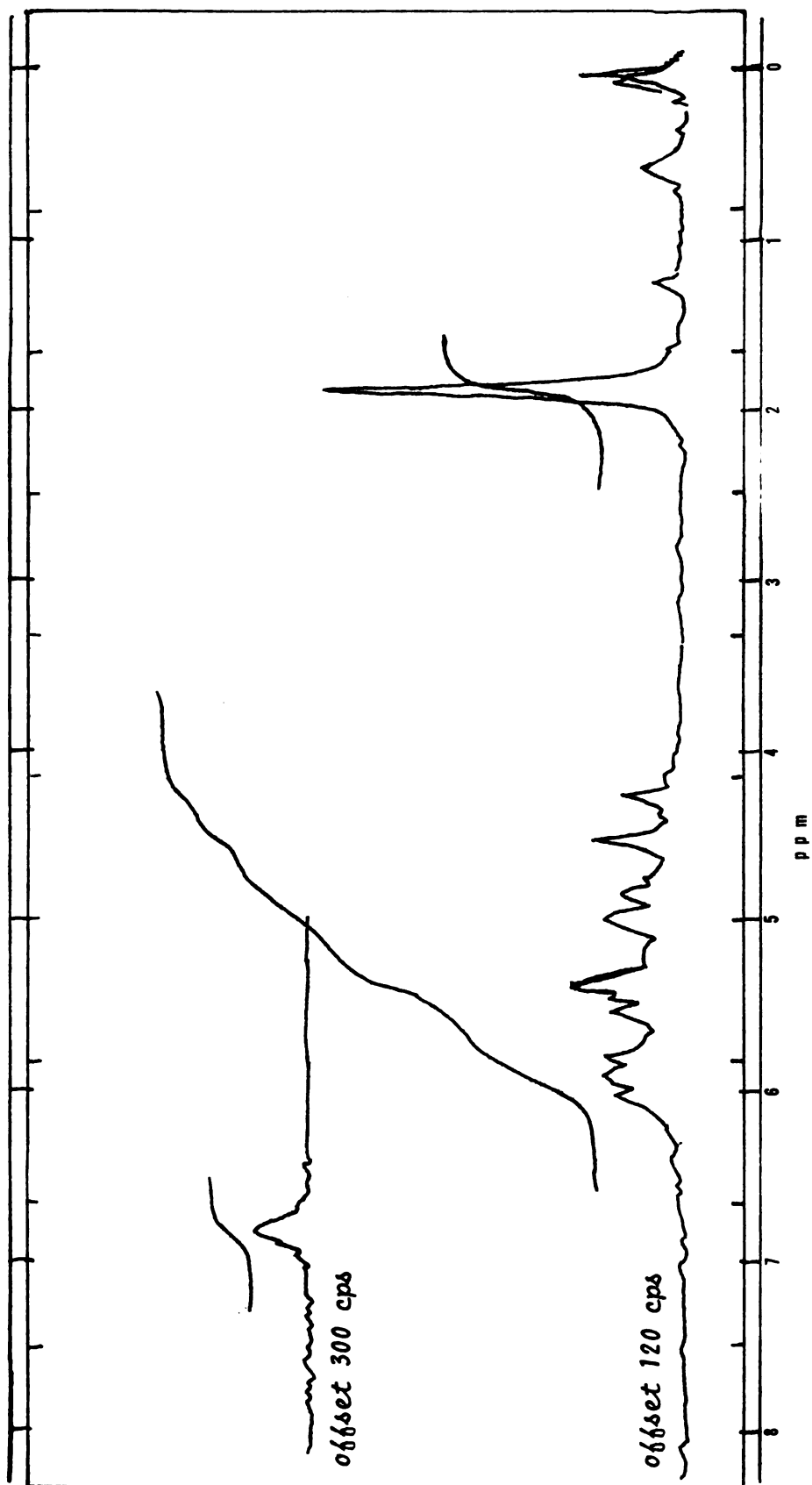
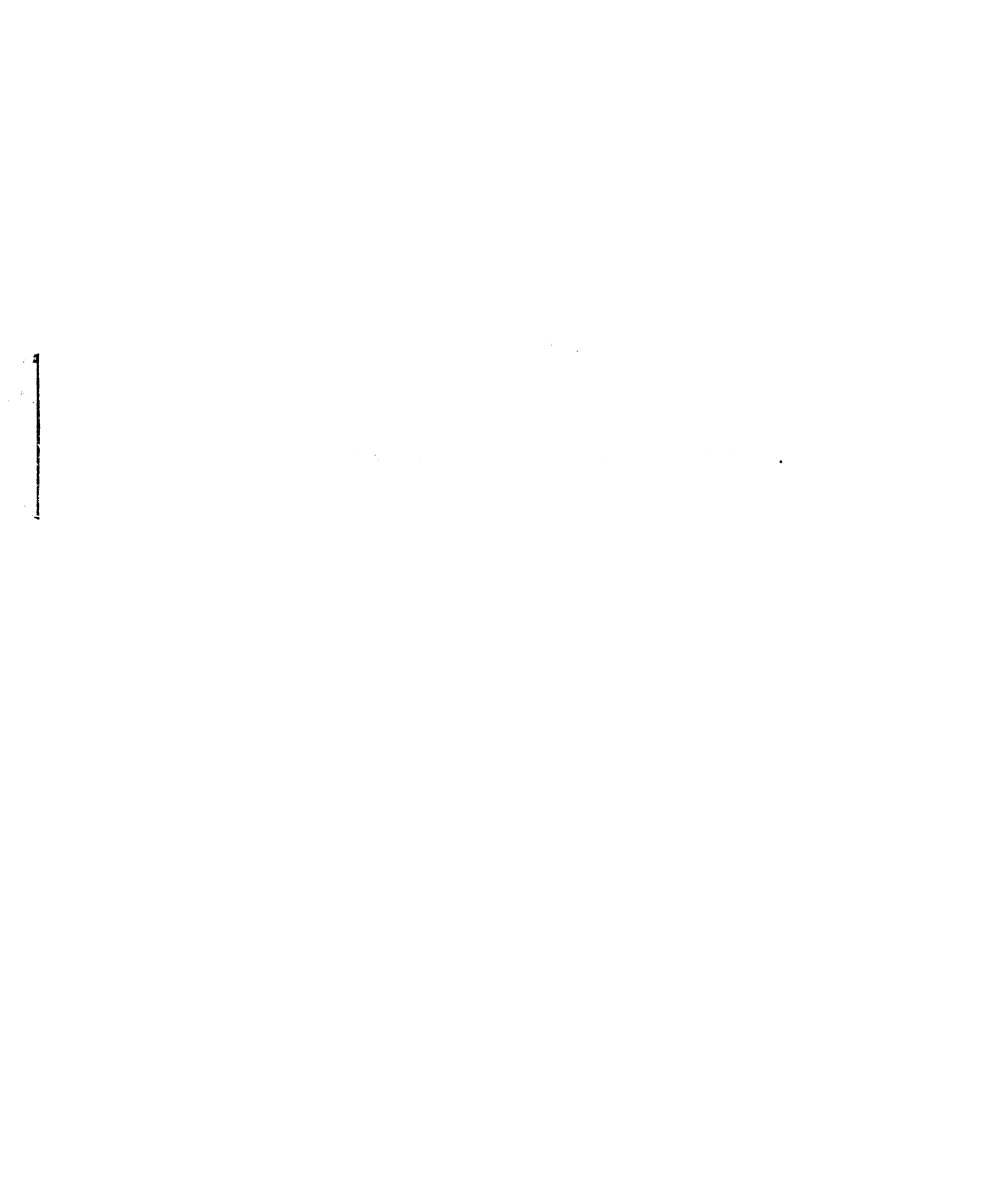


Fig. 17 60 MHz nmr of 5-methoxyindole-3-acrylic acid (in d_6 -DMSO).

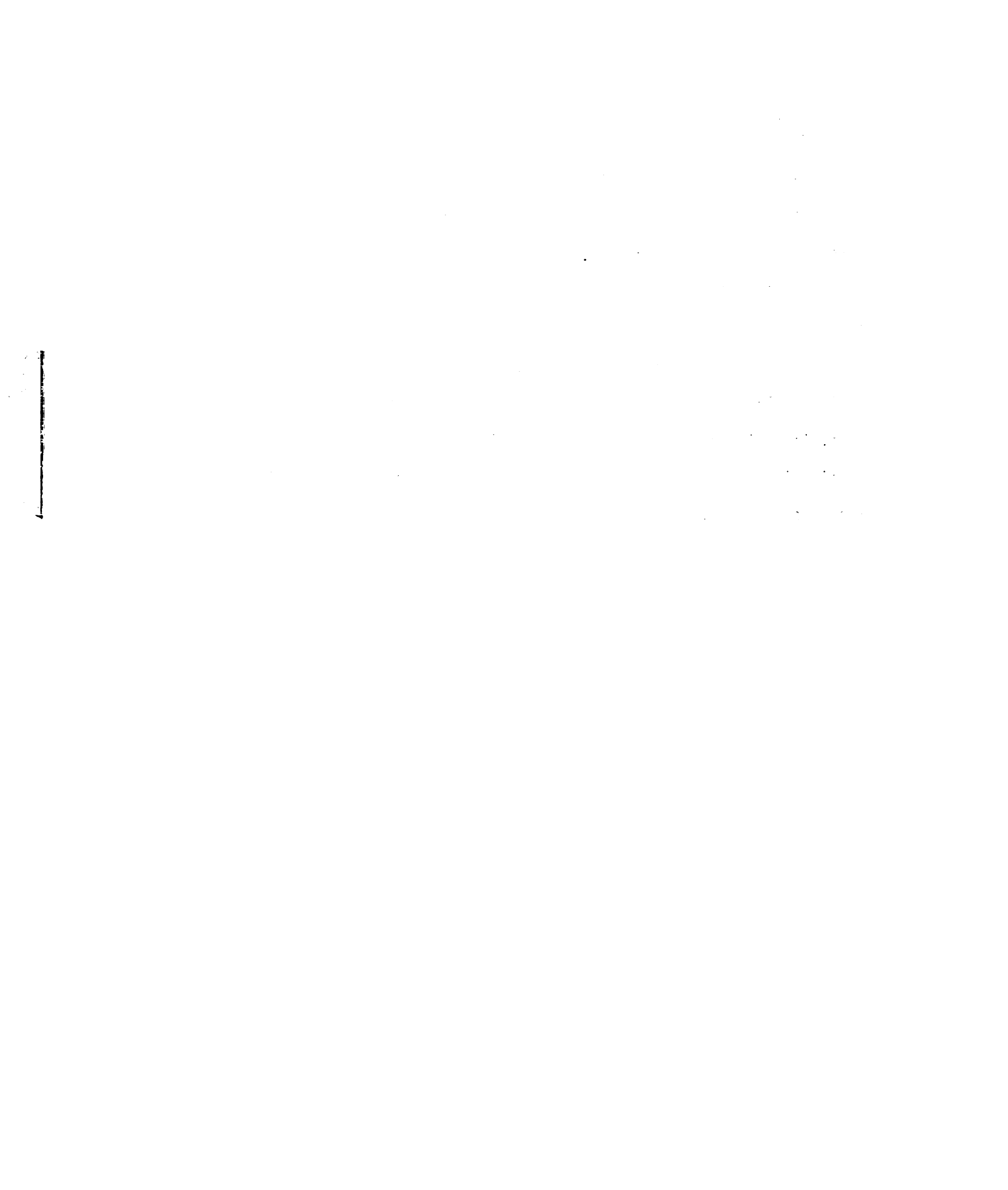
Part II

B. Synthesis of Stable Isotope Labeled Standards



The Preparation of Deuterium Labeled Standards

The preparation of stable isotopically labeled indolic metabolites was essential in my attempt to quantify these metabolites excreted by patients with carcinoid tumors. An isotopically labeled metabolite a few mass units higher in weight than the metabolite under scrutiny would serve as a reference for quantification by comparing peak heights in the chemical ionization mass spectrum. In addition, stable isotopically labeled tryptophan could be used in in vitro and in vivo studies, in a mixture with non-labeled ("cold") tryptophan, to facilitate identification of tryptophan metabolites by the appearance of "doublets" in the mass spectrum.



Tryptophan

The best label would be two mass units higher so as to eliminate any interference from the ^{13}C peak of the "cold" compound. For tryptophan, the easiest positions to place the label during synthesis of the compound are the 5-position of the ring and the 3-position of the side chain (fig. 19). A deuterium can be easily placed on the 5-position by hydrogenolysis of the 5-bromo analog using deuterium gas (92). Two deuteriums can be placed on carbon-3 of the side chain by using d_2 -formaldehyde in the synthesis of tryptophan starting from indole (92). Likewise, use of ^{13}C -formaldehyde will result in ^{13}C incorporation into the 3-position of the side chain. Other positions for ^{13}C incorporation are possible but involve synthesizing the indole nucleus and are therefore more difficult. The ideal label would be two deuteriums on C-3 of the side chain. But the cost of d_2 -formaldehyde or ^{13}C -formaldehyde is prohibitive when one considers that as much as 5 grams of labeled compound would be necessary for a tryptophan load for each patient. Multiply that amount by several patients and controls and the figure goes beyond the resources of the investigation. Therefore, although there were some distinct disadvantages to using 5-deuterio-tryptophan as the labeled compound, cost and ease of preparation made it the only choice.

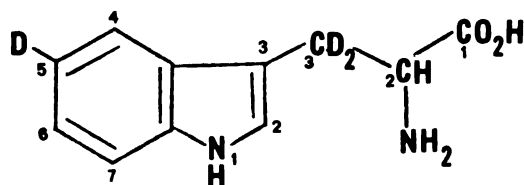


Fig. 19 Tryptophan



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In the second section, the author outlines the various methods used to collect and analyze the data. This includes both manual and automated processes. The goal is to ensure that the data is as accurate and reliable as possible.

The third part of the document provides a detailed breakdown of the results. It shows that there has been a significant increase in sales over the period covered. This is attributed to several factors, including improved marketing strategies and better customer service.

Finally, the document concludes with a series of recommendations for future actions. These include continuing to invest in marketing, improving operational efficiency, and maintaining a strong focus on customer satisfaction.

Some of the disadvantages of using 5-²H-tryptophan can be overcome. It is possible to take into account the ¹³C contribution of the lower mass peak when calculating the ratio of the MH⁺ and MH⁺ +1 peaks for the purpose of quantitation. Also, the problem of loss of deuterium from the 5-position upon hydroxylation is partially solved by the N.I.H. shift. Tritium shifts from the 5-position to the 4-position with 85 per cent retention (93,94). Deuterium would undergo an N.I.H. shift to the 4-position with a probable retention of 75 per cent. Although loss of label may speak against labeling in this position, the loss may be advantageous as only a small amount is lost and this loss may serve to differentiate 5-hydroxylated from non-5-hydroxylated metabolites. The C.I.M.S. of a 5-hydroxylated compound would consist of a "skewed" doublet, the ratio of whose peaks is different from unhydroxylated metabolites.

The synthesis of 5-deuterio-L-tryptophan can best be achieved according to the method of Ek and Witkop (92) (fig. 20). Starting with 5-bromoindole (35), 5-bromogranine (36) was synthesized via a Mannich reaction. Using diethylformamidomalonate in sodium ethoxide, the ester ethyl β-(5-bromoindolyl-3)-α-carbethoxy-λ-formamidopropionate (37) was prepared. Hydrogenolysis was conducted on the ester 37, with D₂ gas and 10% palladium on charcoal, because of the ease of recrystallization of the formamidopropionate ester. Basic hydrolysis of the ester followed by decarboxylation and acid hydrolysis of the amide gave 5-²H-DL-tryptophan (39). The chemical ionization mass spectrum of 39 shows m/e 206 (MH⁺ 5-²H-TP) 79.2% and m/e 205 (MH⁺ 5-²H-TP) 20.8%, with the ¹³C contribution of m/e 205 taken into

205 taken into account. Although this was sufficiently deuterium enriched for the studies I had in mind, the loss of label, down from the 95 per cent incorporation during hydrogenolysis, was puzzling. A certain amount of deuterium is lost from exchangeable positions on the molecule in the chemical ionization process, dependent upon the ease of exchangeability. Deuterium on oxygens and nitrogens are exchanged so rapidly as to render them useless for isotope studies. The 20 per cent loss of label could not be explained by the effects of the chemical ionization process alone. Something in the reaction sequence going from the ester 38 to 5-²H-TP was causing a loss of label. This was the first indication that something was happening to cause loss of label from the 5-position.

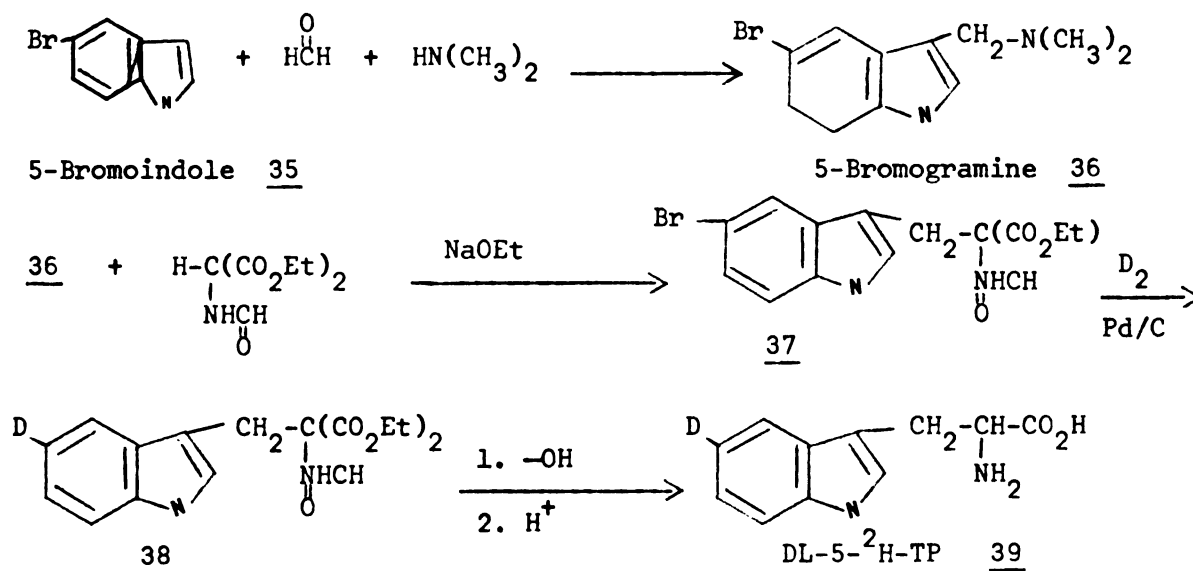
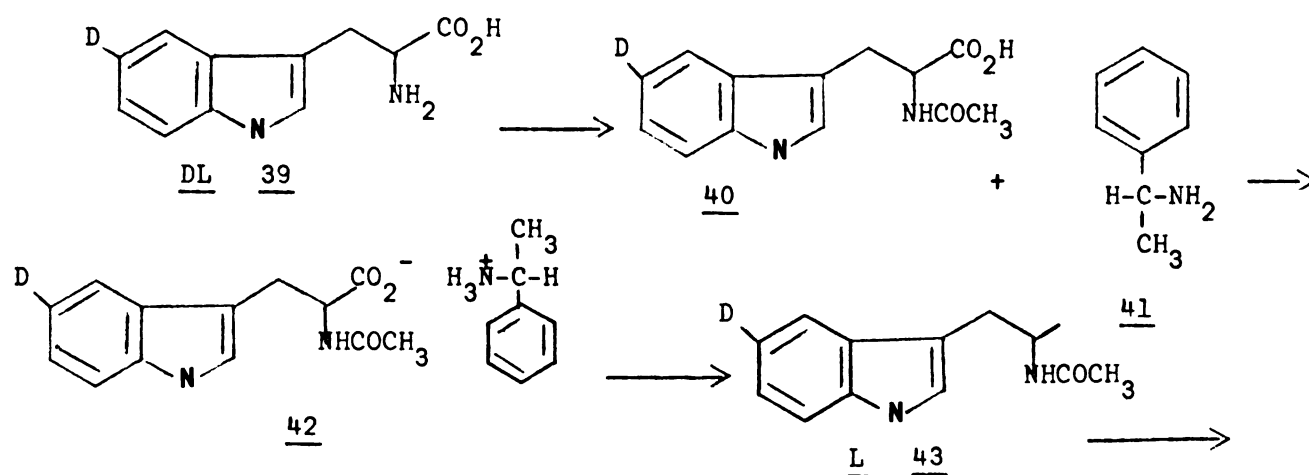


Fig. 20 Synthesis of 5-²H-DL-tryptophan

The resolution of 5-²H-DL-tryptophan to 5-²H-L-tryptophan was accomplished using a procedure that employs the N-acetyl derivative of tryptophan and the amine 1-(-)- α -phenylethylamine (95) (fig. 21).

1

The N-acetyl compound (40) was prepared in 75% yield from the reaction of 5-²H-DL-TP with acetic anhydride in aqueous NaOH. The diastereomeric salt 42 had a rotation of +20.0° in 95% EtOH (lit. + 17.8°) (95). Reflux of free L-5-²H-N-acetyltryptophan (43) in three equivalents HCl gave 5-²H-L-tryptophan (44) that was 75% optically pure. Chemical ionization mass spectral analysis, however, showed only a 22% retention of the 5-deuterium. The loss of 78% of the label could not be explained by loss during chemical ionization.



5-²H-TP
44

Fig. 21 Resolution of 5-²H-DL-tryptophan

Something during the resolution process was causing exchange of the deuterium at position 5 with a proton. It was assumed that the 5-position would be relatively stable and would not exchange under acidic conditions as do protons on the 4-position of 5-hydroxytryptophan. This is the basis for the tritium exchange assay of tryptophan hydroxylase developed by Lovenberg (96). 5-³H-tryptophan is prepared and upon hydroxylation, 4-³H-5-hydroxytryptophan is formed. Upon incubation of the reaction mixture in 6 N perchloric acid

The first part of the document discusses the importance of maintaining accurate records of all transactions. It emphasizes that proper record-keeping is essential for the integrity of the financial system and for the ability to detect and prevent fraud. The text outlines the various methods used to collect and analyze data, including the use of computerized systems and manual audits. It also discusses the challenges of data collection and the need for standardized procedures to ensure consistency and reliability of the information.

CONCLUSION

In conclusion, the document highlights the critical role of data in decision-making and the need for a robust data management strategy. It stresses the importance of data quality, security, and accessibility. The text provides a comprehensive overview of the data management process, from data collection to data analysis and reporting. It also discusses the future of data management and the potential of emerging technologies to revolutionize the way we collect, store, and analyze data. The document serves as a valuable resource for anyone interested in data management and its applications in various industries.

(20 min. at 37°) to catalyze the exchange of tritium from the 4-position of 5-hydroxytryptophan, the HTO formed is counted and thus the activity of the enzyme can be measured as a function of the amount of tritiated water produced. If exchange from the 5-position occurred, false high activity would be reported. It was because the 5-position did not exchange appreciably in 6 N perchloric acid that I assumed it to be relatively stable. However, under the reflux conditions in dilute HCl that are used during the hydrolysis of the N-acetyl group, exchange occurs. A subsequent search of the literature shows this to be the case.

Bak reported in 1967 the complete exchange of the five aromatic protons of tryptophan (H_2 , H_4 , H_5 , H_6 and H_7) when treated with d_1 -trifluoroacetic acid (97). No exchange of the α or β protons of the side chain occurred. The rates were characterized as slow for H_4 , H_5 and H_7 and fast for H_2 and H_6 . Kirby, also in 1967, described the complete exchange of the four aromatic protons (H_2 , H_4 , H_6 and H_7) of 5-hydroxytryptophan when treated with 4 M DCl under reflux for 1 hr (98). Again, no exchange occurred with the α or β side chain protons. The order of rate of exchange was: $4 > 6 > 2 > 7$, B. Bak used his procedure to selectively label tryptophan residues in glucagon with deuterium (99).

I therefore concluded that this was a viable procedure for the preparation of multiply deuterated tryptophan. A solution of L-tryptophan in 2 M DCl was refluxed for 18 hr. The chemical ionization mass spectrum showed m/e 210 ($MH^+ + d_5$) 65% and m/e 209 ($MH^+ + d_4$) 34%. The optical rotation was -31.0° confirming the absence

of any exchange at the α position of the side chain, thus retaining optical purity. Another sample of L-tryptophan was shaken with D_2O to remove any readily exchangeable protons and then treated with d_1 -trifluoroacetic acid. The trifluoroacetic acid solution was stirred under nitrogen atmosphere for 5 hr at room temperature and then the solvent was removed. Fresh d_1 -trifluoroacetic acid was added and the procedure repeated four times, an nmr recording the exchange of aromatic protons after each of the treatments. The maximum obtainable mole ratio of 2,4,5,6,7 - d_5 -L-tryptophan using d_1 -trifluoroacetic acid containing 99+% deuterium is $0.99^5 = 0.95$. Figures 22a,b,c,d, show the nmr after each treatment. After the final treatment (fig. 22d), the aromatic protons integrate for 29 hundredths of a proton. This corresponds to 94.2% exchanged which is close to the maximum amount possible. The optical rotation showed optical purity and the chemical ionization mass spectrum was consistent with approximately 94% d_5 -tryptophan.

1. The first part of the document discusses the importance of maintaining accurate records of all transactions and activities. It emphasizes that this is essential for ensuring transparency and accountability in the organization's operations.

2. The second part of the document outlines the various methods and tools used to collect and analyze data. It highlights the need for consistent and reliable data collection processes to support informed decision-making.

3. The third part of the document focuses on the role of technology in modern data management. It discusses how advanced software solutions can streamline data collection, storage, and analysis, thereby improving efficiency and accuracy.

4. The fourth part of the document addresses the challenges associated with data security and privacy. It stresses the importance of implementing robust security measures to protect sensitive information from unauthorized access and breaches.

5. The fifth part of the document provides a summary of the key findings and recommendations. It concludes that a comprehensive data management strategy is crucial for the long-term success and growth of the organization.

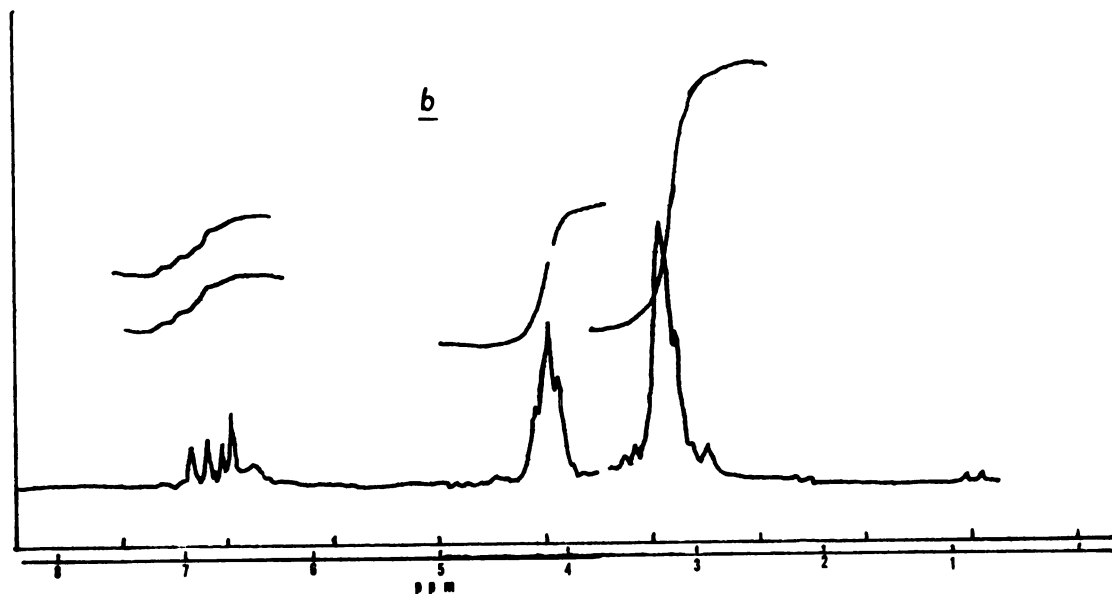
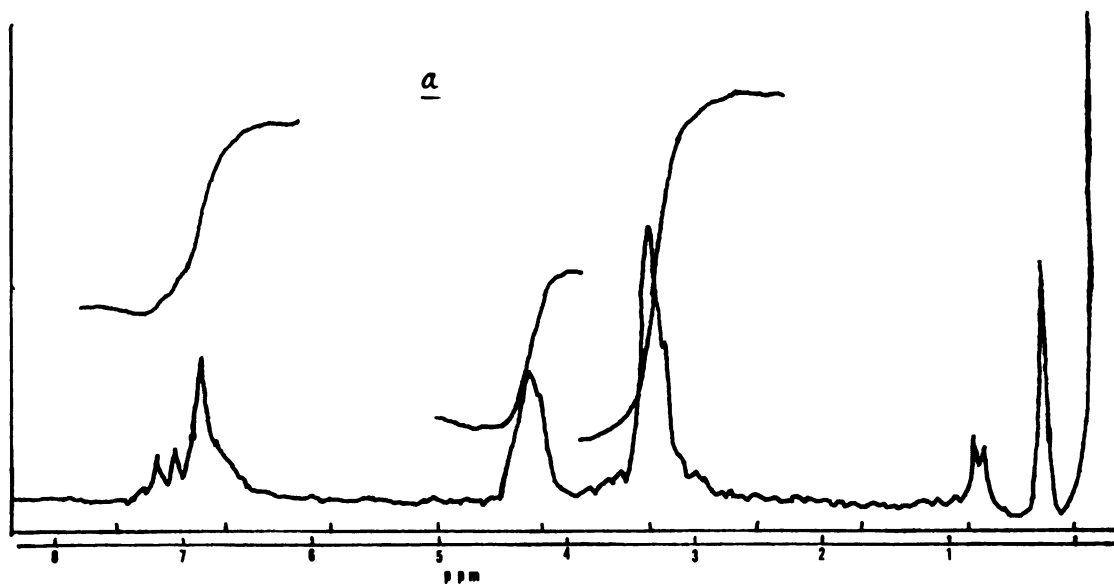


Fig. 22a TOP 60 MHz nmr of L-tryptophan after one treatment with d_7 -trifluoroacetic acid. (in d_7 -TFAA)

Fig. 22b Bottom 60 MHz nmr of L-TP after two treatments with d_7 -TFAA. (in d_7 -TFAA)

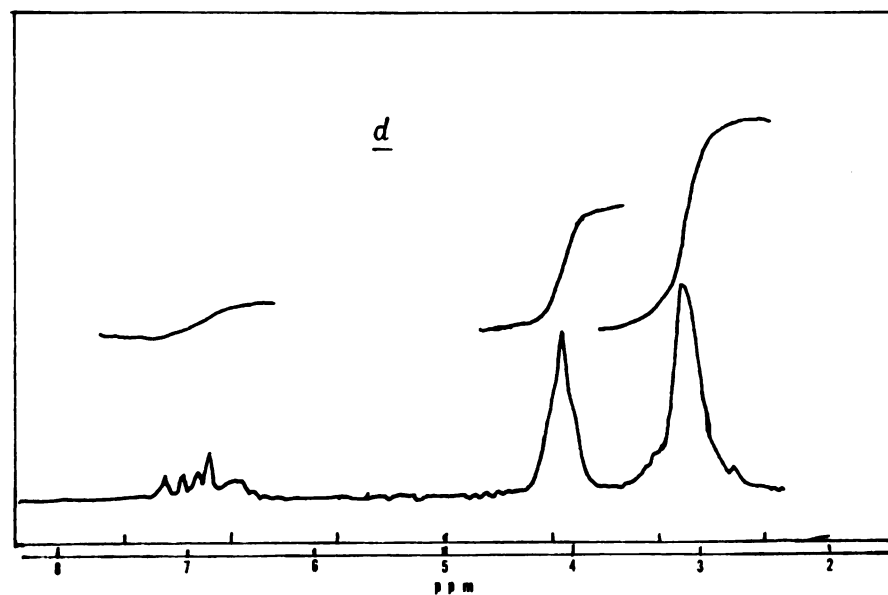
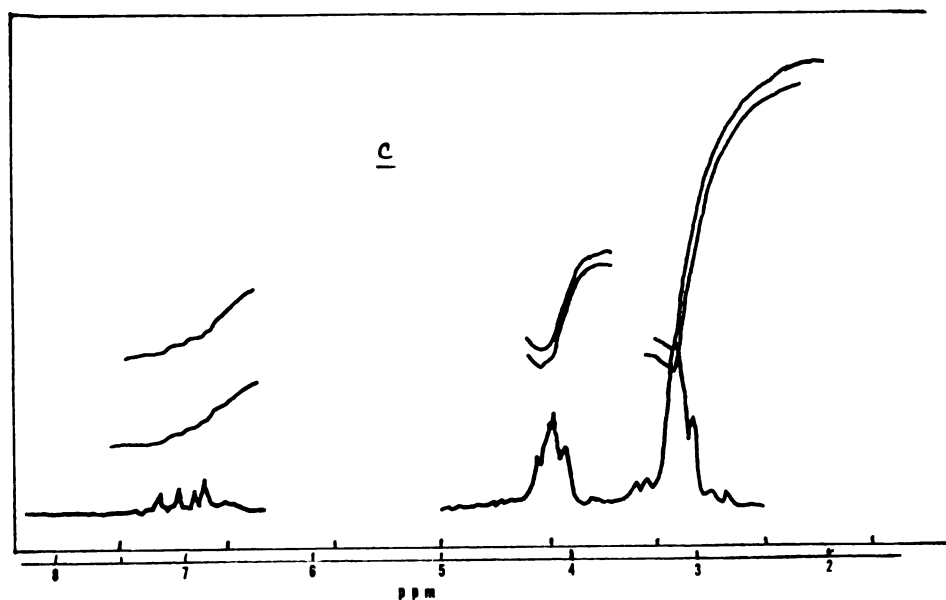


Fig. 22c TOP 60 MHz nmr of L-TP after three treatments with d_7 -TFAA. (in d_7 -TFAA)

Fig. 22d Bottom 60 MHz nmr of L-TP treated four times with d_7 -TFAA. (in d_7 -TFAA)

Tryptophol and 5-Hydroxytryptophol

Introduction

Among the metabolites of tryptophan that are of particular interest in disease states are tryptophol (TOH) and 5-hydroxytryptophol (5-HTOH). As seen in figure 23, tryptophol and 5-hydroxytryptophol are produced when tryptamine and 5-hydroxytryptamine are oxidized to the intermediate aldehyde by monoamine oxidase and then reduced to the alcohol rather than oxidized to the acid. 5-Hydroxytryptophol and its conjugates have been identified as normal metabolites of 5-HT in human urine and human and rat brain homogenates (101,102). However, a greater shift to the reductive pathway can occur. This shift to the reduced metabolite can be caused by ethanol consumption. Studies in humans show a dose-response relationship between the amount of alcohol ingested and the decrease in urinary excretion of 5-HIAA (103). Further human studies demonstrated that the pattern of ^{14}C serotonin metabolites excreted in an 8 hour urine collection following the ingestion of ^{14}C serotonin was markedly changed by prior ingestion of ethanol (103). After alcohol ingestion, the per cent of the total ^{14}C excreted as 5-HIAA decreased from 82 to 42 while the per cent excreted as 5-hydroxytryptophol and its conjugates rose from 2 to 42. It was considered likely that this shift of serotonin metabolites from an oxidative pathway to a reductive pathway reflects the increased NADH/NAD ratio effected by ethanol metabolism (104). This alteration in serotonin metabolism was demonstrated in normal subjects and in patients with carcinoid tumors. In patients with the carcinoid syndrome and markedly high levels of 5-HT production, alcohol ingestion could readily

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2. The second part of the document focuses on the implementation of robust risk management strategies. It outlines various risk assessment techniques and provides guidance on how to identify, measure, and mitigate potential risks. The text stresses the need for a proactive approach to risk management to protect the organization's assets and reputation.

3. The third part of the document addresses the importance of effective communication and reporting. It discusses the need for clear and concise communication channels and the role of regular reporting in keeping stakeholders informed. This section also touches upon the importance of maintaining accurate financial statements and providing timely updates to management and investors.

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9. The ninth part of the document addresses the importance of effective communication and reporting. It discusses the need for clear and concise communication channels and the role of regular reporting in keeping stakeholders informed. This section also touches upon the importance of maintaining accurate financial statements and providing timely updates to management and investors.

10. The tenth part of the document discusses the importance of maintaining accurate records of all transactions and activities. It emphasizes that proper record-keeping is essential for ensuring transparency and accountability in financial operations. This section also highlights the role of internal controls in preventing fraud and errors.

result in greatly increased and prolonged levels of 5-hydroxyindoleacetaldehyde and 5-hydroxytryptophol (105). Thus, I felt it necessary to assay for 5-HTOH and the non-hydroxylated metabolite, tryptophol, in order to further account for the metabolites of tryptophan.

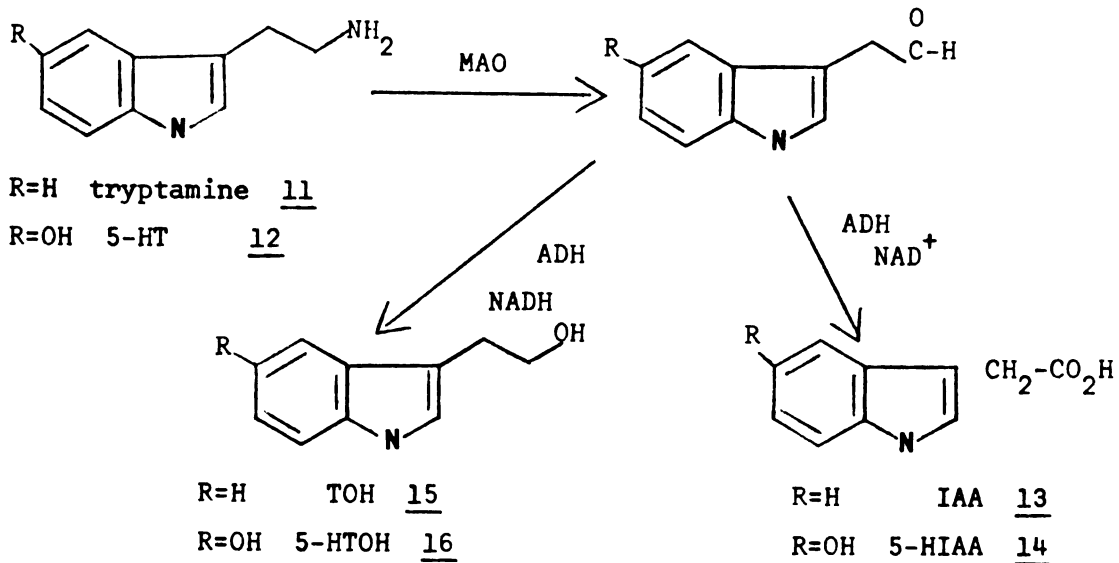


Fig. 23 Metabolic formation of tryptophol and 5-hydroxytryptophol

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Preparation of d_4 -Tryptophol and d_4 -5-Hydroxytryptophol

The presence of the ethyl side chain conveniently allowed preparation of compounds labeled with four deuterium atoms. d_4 -Tryptophol was prepared according to the procedure for tryptophol of Nogrady and Doyle (106). Ethyl 3-indolylglyoxylate (48) was prepared by reacting indole with oxalyl chloride and treating the resulting 3-indolylglyoxylyl chloride (46) with ethanol in the presence of triethylamine (fig. 24). The ester was then reduced with lithium aluminum deuteride in THF to give d_4 -tryptophol (49).

d_4 -5-Hydroxytryptophol was prepared according to the procedure for 5-hydroxytryptophol of Kveder, et al (107). 5-Benzyloxyindole-3-glyoxylyl chloride (47) was prepared by reacting 5-benzyloxyindole with oxalyl chloride. The acid chloride (47) is suitable for reduction with hydride and treatment with lithium aluminum deuteride in THF gave d_4 -5-benzyloxytryptophol (50). This product was treated with 10% Pd/BaSO₄ under an atmosphere of H₂ to give d_4 -5-hydroxytryptophol (51).

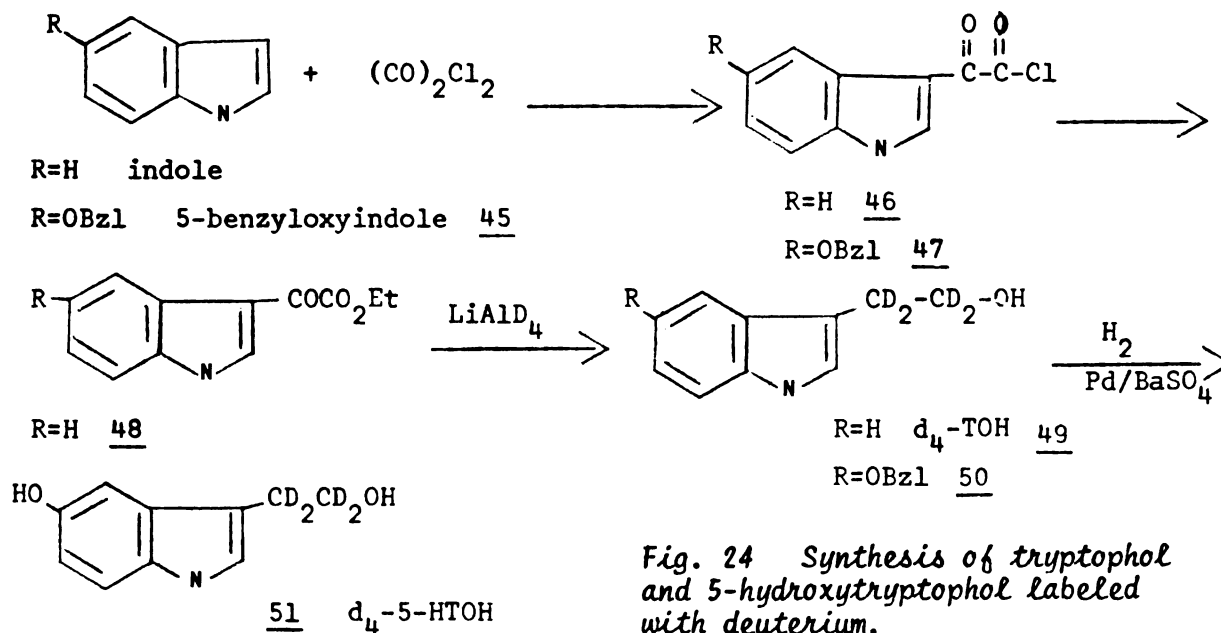


Fig. 24 Synthesis of tryptophol and 5-hydroxytryptophol labeled with deuterium.

1. The first part of the document discusses the importance of maintaining accurate records of all transactions and activities.

2. It is essential to ensure that all data is entered correctly and consistently to avoid any discrepancies or errors.

3. Regular audits and reviews should be conducted to verify the accuracy and integrity of the information.

4. The use of standardized procedures and protocols is crucial for maintaining the reliability of the data.

5. It is also important to ensure that all personnel involved in the process are properly trained and informed.

6. The document further outlines the necessary steps for handling any identified issues or discrepancies.

7. Finally, it emphasizes the need for ongoing communication and collaboration between all stakeholders.

8. The overall goal is to ensure that the data is accurate, reliable, and up-to-date at all times.

9. This approach will help to minimize the risk of errors and ensure the highest quality of data.

10. The document concludes by reiterating the importance of these practices for the success of the project.

11. It is hoped that these guidelines will be followed to the letter and that the project will be completed successfully.

12. Thank you for your attention and cooperation in this matter.

Indoleacetic Acid

The observation that the side chain protons of tryptophan do not exchange in acid does not hold true for the side chain protons of indoleacetic acid and 5-hydroxyindoleacetic acid. The side chain protons in these two compounds are not only benzylic (indolylic) but are α to a carbonyl thus increasing their acidity. A solution of IAA in $D_2O + DCI$ was refluxed for several hours, the solvent was removed and fresh solvent added. After repeating this procedure five times, the nmr (fig. 25b) showed no aromatic protons, just the protons of the side chain. Table 5 shows the per cent incorporation of deuterium expressed as relative peak heights.

Table 5

The relative peak heights of a chemical ionization mass spectrum of IAA treated six times with DCI in D O. (Corrected for ^{13}C contribution) (methylester derivative)²

<u>mass</u>	<u>no. of deuteriums</u>	<u>relative abundance</u>	<u>%</u>
189	d ₀	-	0
190	d ₁	-	0
191	d ₂	-	0
192	d ₃	-	0
193	d ₄	26.8	11.3
194	d ₅	100	42.1
195	d ₆	83.5	35.1
196	d ₇	27.5	11.5

The first part of the document discusses the importance of maintaining accurate records of all transactions and activities. It emphasizes that proper record-keeping is essential for ensuring transparency and accountability in the organization's operations. The text outlines various methods and tools that can be used to collect, store, and analyze data effectively. It also highlights the need for regular audits and reviews to identify any discrepancies or areas for improvement. The second part of the document focuses on the role of technology in streamlining processes and enhancing efficiency. It explores different software solutions and digital tools that can be implemented to automate repetitive tasks and improve communication. The text also discusses the importance of data security and privacy, providing guidelines on how to protect sensitive information from unauthorized access. Finally, the document concludes by summarizing the key points and offering recommendations for future actions. It encourages the organization to embrace a culture of continuous learning and innovation to stay competitive in the ever-changing market.

Item	Description	Quantity	Unit Price	Total Value
1	Office Supplies	50	\$2.00	\$100.00
2	Travel Expenses	10	\$15.00	\$150.00
3	Marketing Costs	20	\$10.00	\$200.00
4	Professional Fees	5	\$30.00	\$150.00
5	Utilities	12	\$12.50	\$150.00
6	Insurance	3	\$50.00	\$150.00
7	Salaries	100	\$10.00	\$1,000.00
8	Rent	1	\$1,000.00	\$1,000.00
9	Depreciation	1	\$500.00	\$500.00
10	Interest	1	\$200.00	\$200.00
11	Income Tax	1	\$100.00	\$100.00
12	Retirement	1	\$50.00	\$50.00
13	Charitable Contributions	1	\$25.00	\$25.00
14	Gifts	1	\$10.00	\$10.00
15	Entertainment	1	\$5.00	\$5.00
16	Travel	1	\$10.00	\$10.00
17	Meals	1	\$5.00	\$5.00
18	Transportation	1	\$5.00	\$5.00
19	Medical	1	\$5.00	\$5.00
20	Education	1	\$5.00	\$5.00
21	Legal	1	\$5.00	\$5.00
22	Accounting	1	\$5.00	\$5.00
23	Insurance	1	\$5.00	\$5.00
24	Utilities	1	\$5.00	\$5.00
25	Travel	1	\$5.00	\$5.00
26	Meals	1	\$5.00	\$5.00
27	Transportation	1	\$5.00	\$5.00
28	Medical	1	\$5.00	\$5.00
29	Education	1	\$5.00	\$5.00
30	Legal	1	\$5.00	\$5.00
31	Accounting	1	\$5.00	\$5.00
32	Insurance	1	\$5.00	\$5.00
33	Utilities	1	\$5.00	\$5.00
34	Travel	1	\$5.00	\$5.00
35	Meals	1	\$5.00	\$5.00
36	Transportation	1	\$5.00	\$5.00
37	Medical	1	\$5.00	\$5.00
38	Education	1	\$5.00	\$5.00
39	Legal	1	\$5.00	\$5.00
40	Accounting	1	\$5.00	\$5.00
41	Insurance	1	\$5.00	\$5.00
42	Utilities	1	\$5.00	\$5.00
43	Travel	1	\$5.00	\$5.00
44	Meals	1	\$5.00	\$5.00
45	Transportation	1	\$5.00	\$5.00
46	Medical	1	\$5.00	\$5.00
47	Education	1	\$5.00	\$5.00
48	Legal	1	\$5.00	\$5.00
49	Accounting	1	\$5.00	\$5.00
50	Insurance	1	\$5.00	\$5.00

Exchange of the aromatic protons was 89% complete while the side chain protons were just beginning to be exchanged. The maximum obtainable mole ratio of $\alpha,\alpha,2,4,5,6,7\text{-d}_7\text{-IAA}$ using 99+% DCl is $0.97 = 0.91$.

Following the procedure of Kirby (98), the incompletely deuterated IAA was refluxed in 4.0 M DCl in D₂O for 1 hr. The chemical ionization mass spectrum (table 6) showed the exchange with deuterium was 22% complete.

Table 6

The relative peak heights of a chemical ionization mass spectrum of IAA treated seven times with DCl in D₂O. (Corrected for ¹³C contribution.) (Methyl ester derivative)²

<u>mass</u>	<u>no. of deuteriums</u>	<u>relative abundance</u>	<u>% ion current</u>
189-192	d ₀ -d ₃	none	-
193	d ₄	11.0	5.0
194	d ₅	62.0	28.0
195	d ₆	100	45.1
196	d ₇	48.5	21.9

Treating the deuterated compound twice more with 4 M DCl gave 65% d₇-IAA and 29% d₆-IAA, (table 7) which was sufficient deuteration so as to enable me to use this compound for a mass spectral assay. The use of d₇ -IAA in the C.I.M.S. assay is described in the section dealing with the assay procedure.

I found this procedure to be unsatisfactory for preparation of d₆-5-HIAA due to the greater instability of 5-HIAA in strong acid. The decomposition was great enough to significantly reduce the recovery of the deuterated compound. Another method of deuteration had to be developed.

Table 7

The relative peak heights of a chemical ionization mass spectrum of IAA treated nine times with DCl in D₂O. (methyl ester derivative) (Corrected for ¹³C contribution).

<u>Mass</u>	<u>no. of deuteriums</u>	<u>relative abundance</u>	<u>% ion current</u>
189-193	d ₀ -d ₄	none	-
194	d ₅	9	5.8
195	d ₆	45	29.4
196	d ₇	100	64.7

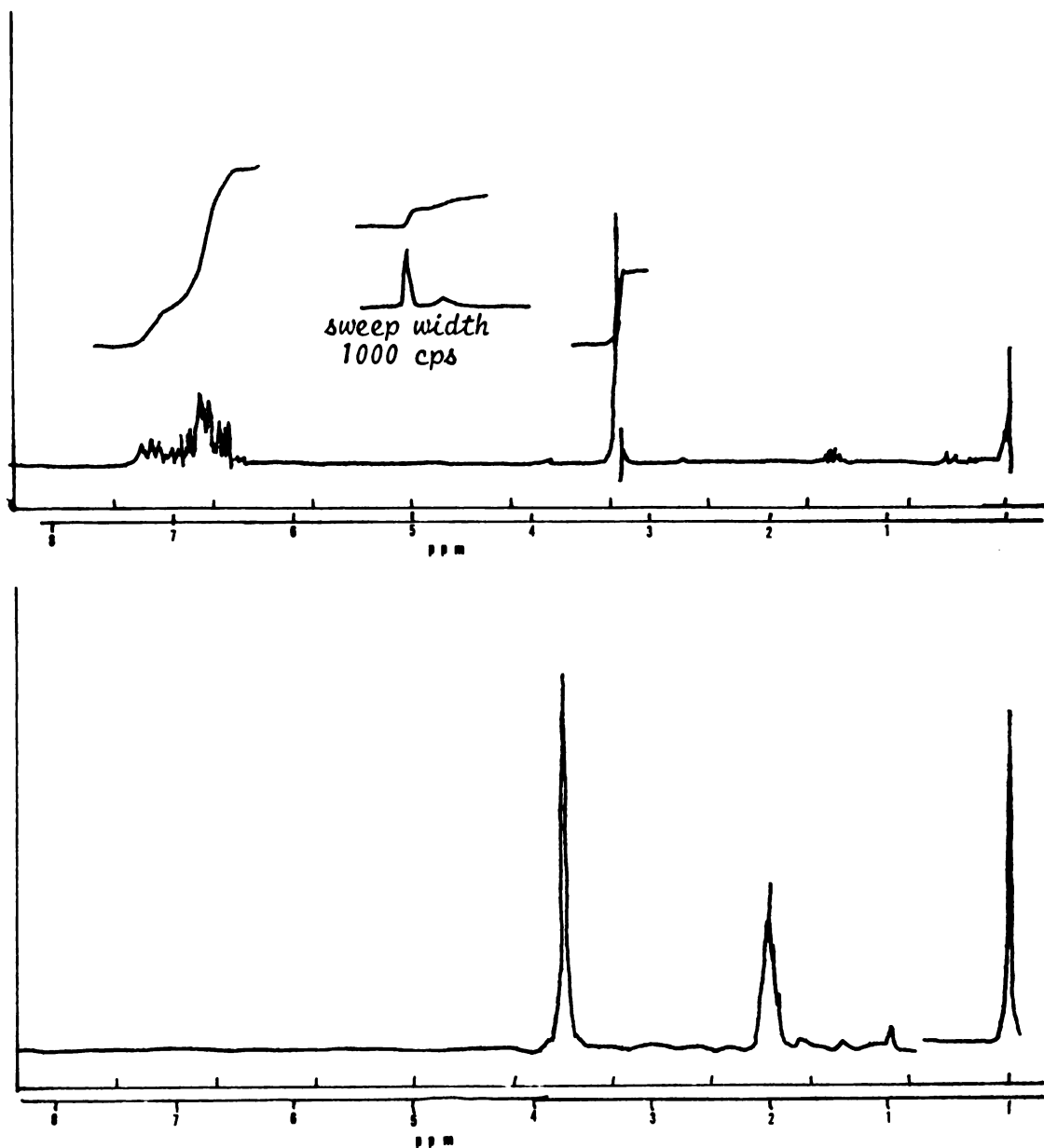


Fig. 25TOP _a 60 MHz nmr of IAA (in d_6 -acetone).

Fig. 25b Bottom 60 MHz nmr of IAA after treatment with DCl in D_2O under reflux (in d_6 -acetone).

Part II

C. Experimental

Experimental

3,5-di-t-butyl-1,2-benzoquinone (18)

A solution of 3,5-di-t-butylcatechol (10g, 0.045 mol), PbO_2 (24 g, 0.10 mol) and Na_2SO_4 (20 g) in 50 ml ether was stirred at -10 to -5° for 10 min. The reaction mixture was filtered and the filtrate concentrated in vacuo. The resulting crude quinone was recrystallized from absolute EtOH to give massive red prisms, 8.9 g (90%). NMR (CDCl_3) δ 1.23 (s, 9H, 5-t-butyl), 1.27 (s, 9H, 3-t-butyl), 6.24 (d, 1H, 4-H), 6.98 (d, 1H, 6-H), $J_{4,5} = 2.5$ cps.

Cylchexanone

To a solution of cyclohexylamine (116 mg, 1.18 mmol) in 3 ml MeOH was added 3,5-di-t-butyl-1,2-benzoquinone (219 mg, 0.99 mmol) in 4 ml MeOH. The mixture was stirred at room temperature for 5 hr under N_2 atmosphere. After 20 min the reaction mixture changed from deep red to green and was clear after 5 hr at which time 7 ml THF and 3 ml H_2O were added and the pH was adjusted to 4.1 by addition of oxalic acid dihydrate. Hydrolysis was allowed to continue for 4 1/2 hrs and then the reaction mixture was extracted with ether/pentane (1:1). The organic extracts were washed with saturated NaCl solution, dried over Na_2SO_4 and concentrated in vacuo to give an oil, 72.4 mg (74%). The 2,4-dinitro phenylhydrazone derivative was prepared: mp $160-163^\circ$ (lit. 162°) (67).

Acetophenone

To a solution of α -phenylethylamine (0.94 g, 7.77 mmol) in 3 ml MeOH was added 3,5-di-*t*-butyl-1,2-benzoquinone (1.0g, 4.55 mmol) in 14 ml MeOH and the mixture was stirred at room temperature under N_2 atmosphere. After 19 hr, 42 ml THF and 14 ml H_2O were added, the pH was adjusted to 4.2 by addition of oxalic acid dihydrate and hydrolysis was allowed to continue for 1 1/2 hr. The reaction mixture was then diluted with 80 ml H_2O and extracted with ether/pentane (1:1). The organic extracts were washed with 0.1 N HCl, saturated NaCl solution, dried over Na_2SO_4 and concentrated in vacuo to give an oil. Distillation gave pure acetophenone, 294 mg (54%): b.p. 198-201° (lit. 200°) (67). The 2,4-dinitrophenylhydrazone derivative was prepared: mp 242-243° (lit. 250°) (67).

DL-5-Hydroxytryptophan Methers Ester HCl

A solution of 5-hydroxytryptophan (1.0g, 4.5 mmol) was added to a mixture of 20 ml MeOH and 0.7 ml thionyl chloride at -5° to 0° with constant stirring. The reaction mixture was stirred at -5° to 0° for 1 hr under N_2 atmosphere and an additional 48 hr at room temperature. The solvent was then removed in vacuo and the residue recrystallized from EtOH/ H_2O to give a white power, 962 mg (78%): mp 206-7° NMR (CD_3OD) δ 3.3 (d, 2H, β -H), 3.8 (s, 3H, o-methyl), 4.3 (t, 1H, α -H), 6.7-7.3 (m, 4H, 2-H, 4-H, 6-H, 7-H).

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2-Methyl-4-[3'-(5'-acetoxy)-indolal]-5-oxazolone (28)

A solution of DL-5-hydroxytryptophan (2.2 g, 0.01 mol) (Pierce Chemical Co.) in 30 ml 1.0N NaOH and 50 ml H₂O was stirred at 0-5° as chloroacetyl chloride (2.26 g, 0.02 mol) was added dropwise over 1 hr. The pH was maintained between 10-11 by concomitant addition of 1.0 N NaOH. The solution was stirred for 10 min more, 100 ml EtOAc was added, and the mixture was acidified to pH 2.0 with 1.0 N HCl. The aqueous phase was separated and extracted with EtOAc. The EtOAc extracts were combined, dried over MgSO₄ and concentrated to dryness in vacuo to give the crude N-chloroacetyl compound (26). The crude residue was dissolved in 2.4 ml pyridine and 7.5 ml acetic anhydride and the mixture was allowed to stand for 20 min with occasional stirring 26. 26 dissolved rapidly and the temperature rose. The wine-red solution was poured over 100 ml crushed ice and the brownish-yellow precipitate of 28 was collected and dried in vacuo over P₂O₅ and KOH to give 2.15 g (76%): mp 148-157°. Recrystallization of the crude product three times from benzene gave a bright yellow powder, 1.31 g (46%): mp 210-215°. NMR (CD₃OD) δ 2.3 (2s, 6-9 H, -COCH₃), 6.85-8.4 (m, 5H, 2'-H, 4'-H, 6'-H, 7'-H, indolal-H). Mass spectrum (electron impact) m/e 284 (M⁺), m/e 326 (M⁺+42).

5-Hydroxyindolepyruvic acid

A solution of the oxazolone 28 (325 mg, 1.14 mmol) in 25 ml 2.0 N NaOH was refluxed for 6 hr under N₂ atmosphere in the dark. During the reaction, NH₃ was evolved and after 6 hr evolution ceased. The reaction mixture was cooled to 5° and 60 g crushed ice and 25 ml

ether were added. The solution was acidified to pH 2.8 as rapidly as possible with addition of 1.0 N HCl. The aqueous layer was separated and extracted with several volumes of EtOAc. The EtOAc extracts were combined with the ether phase, dried over MgSO_4 and concentrated to a dark residue in vacuo. The residue was suspended in acetone, filtered, treated with charcoal and benzene was added to give an acetone-benzene ratio of 1:3. After 2 days at 5°, a brown powder was collected, 30.3 mg (12%) mp 209-215°. Mass spectrum (electron impact) m/e 191 ($\text{M}^+ - \text{CO}$), chemical ionization mass spectrum (isobutane) (after treating with diazomethane): m/e 248 (MH^+).

the 1990s, the number of people living in poverty in the United States increased from 35 million to 40 million. The number of children living in poverty increased from 12 million to 15 million. The number of people living in poverty in the United States is now higher than in any other country in the world. The number of children living in poverty in the United States is now higher than in any other country in the world. The number of people living in poverty in the United States is now higher than in any other country in the world. The number of children living in poverty in the United States is now higher than in any other country in the world.

5-Methoxyindole-3-acrylic Acid (32)

A solution of 5-methoxyindole (2.94 g, 0.02 mol) in 20 ml Et₂O was added dropwise, over a period of 15 min, to a pre-packaged solution of methyl magnesium bromide (2.77 g, 0.025 mol) in 10 ml Et₂O under N₂ atmosphere cooled to -5 to 0° in an ice bath. After stirring for 15 min, a solution of 3-chloroacrylic acid (2.13 g, 0.02 mol) in 20 ml Et₂O was added dropwise, over a period of 15 min. The reaction mixture was allowed to warm to room temperature and ammonium chloride (2.8 g) in 30 ml H₂O was added dropwise and the precipitate removed by filtration. The filtrate was separated and the aqueous phase, pH 7.8, was extracted with ether. The pH of the aqueous phase was adjusted to 1.6 by addition of 6N HCl, cooled and the white precipitate collected. Recrystallization first from EtOAc-Hexane (1:1) with charcoal treatment and then from EtOH-H₂O gave 32 as white platelets, analytically pure, 259 mg (6%): mp 197-200°; NMR (DMSO) δ 6.4, 7.9 (2d, 2H, -CH=CH-, J=15 Hz), 6.8-8.0 (m, 5H, N-H, 2-H, 4-H, 6-H, 7-H), 11.85 (s, CO₂H), 3.8 (s, 3H, OCH₃). Chemical ionization mass spectrum (isobutane) of methyl ester m/e 232 (MH⁺). Anal. calcd. for C₁₂H₁₁NO₃: C, 66.3; H, 5.1; N, 6.4. Found: C, 66.18; H, 4.97; N, 6.53.

1. The first step in the process of identifying a problem is to recognize that a problem exists. This is often done by comparing current performance with a desired state or goal. For example, a manager might notice that sales are declining or that customer satisfaction is low. Once a problem is identified, the next step is to define it more precisely. This involves determining the scope of the problem, its causes, and its effects. For instance, a manager might define a problem as "a 10% decrease in sales over the last quarter, primarily due to a loss of market share in the competitive market." This definition helps to narrow down the focus of the problem and provides a clear starting point for further investigation.

2. The second step in the process is to gather information about the problem. This involves collecting data and facts that are relevant to the problem. For example, a manager might gather data on sales trends, market conditions, and customer feedback. This information is then used to identify the underlying causes of the problem. For instance, a manager might discover that the loss of market share is due to a combination of factors, including increased competition, changes in consumer preferences, and a lack of innovation in the product line. This information is crucial for developing an effective solution.

3. The third step in the process is to analyze the information and identify the root cause of the problem. This involves using logical reasoning and problem-solving techniques to determine the underlying factors that are contributing to the problem. For example, a manager might use a fishbone diagram to identify the root cause of the sales decline. This diagram helps to visualize the relationship between different factors and their impact on the problem. In this case, the root cause might be identified as a lack of innovation in the product line, which is leading to a loss of market share.

4. The fourth step in the process is to develop a solution. This involves identifying a course of action that will address the root cause of the problem and restore performance to the desired state. For example, a manager might develop a solution that involves investing in research and development to create new products, improving marketing efforts, and enhancing customer service. This solution is then implemented and monitored to ensure that it is effective in addressing the problem.

5. The fifth and final step in the process is to evaluate the solution and determine its effectiveness. This involves comparing current performance with the desired state and assessing the impact of the solution. For example, a manager might track sales trends and customer satisfaction over time to determine if the solution has been effective in addressing the problem. If the solution is found to be effective, the manager can then implement it on a larger scale. If the solution is found to be ineffective, the manager can then return to the first step and re-evaluate the problem.

5-Bromogramine (36)

A solution of 5-bromoindole (78.8 g, 0.4 mol) in 200 ml dioxane was added dropwise over two hr to an ice-cooled, stirred solution of formaldehyde (12.3 g, 0.41 mol), aq. dimethylamine (22.05 g, 0.49 mol) and acetic acid (300 ml) in 250 ml dioxane. The reaction mixture was stirred under N₂ atmosphere with cooling and with the exclusion of light for 2 hr and then allowed to warm to room temperature overnight whereupon 400 ml H₂O was added. The reaction mixture was treated with charcoal, filtered and the filtrate pH adjusted to 12. The resulting white precipitate was collected, washed with H₂O and recrystallized from benzene with charcoal treatment giving 70.2 g (70%): mp 156-157° (lit. 162°) (68).

Ethyl β-(5-bromoindolyl-3)-α-carbethoxy-α-formamidopropionate (37)

A mixture of diethylformamidomalonate (9.1 g, 0.045 mol) and powdered NaOH (0.8 g, 0.02 mol) in 100 ml toluene was stirred while 5-bromogramine (10.12 g, 0.04 mol) was added. The reaction mixture was then refluxed for 6 hr while a vigorous stream of N₂ was bubbled through. The mixture was then filtered hot, allowed to cool and concentrated in vacuo to 50 ml. After cooling overnight, the fine white precipitate was collected, washed with toluene and benzene and dried in vacuo to give 15.0 g (91%): mp 125-130°. A small sample was recrystallized from EtOH (95%) to give fine, white, analytically pure needles: mp 134-135°. Anal. (C₁₇H₁₉N₂O₅Br) Calc. for C, 49.6; H, 4.6; N, 6.8; Br, 19.5. Found: C, 49.57; H, 4.58; N, 6.88; Br, 19.6.

The first part of the document discusses the importance of maintaining accurate records of all transactions and activities. It emphasizes the need for transparency and accountability in financial reporting. The second part outlines the various methods used to collect and analyze data, including surveys, interviews, and focus groups. The third part presents the findings of the study, highlighting key trends and insights. The final part concludes with recommendations for future research and practical applications of the findings.

The study was conducted over a period of six months, during which a total of 150 participants were interviewed. The data collected was analyzed using a combination of quantitative and qualitative methods. The results show that there is a significant correlation between the variables studied, and that the findings have important implications for the field. The study also identifies several areas for further research, including the need for more comprehensive data collection and the development of more effective analytical tools.

Ethyl β -(5-²H-indolyl-3)- α -carbethoxy- α -formamidopropionate (38)

To a suspension of 75 mg 10% palladium on charcoal in 100 ml EtOAc that has already been saturated with D₂ gas was added the formamidopropionate ester (6.2 g, 0.015 mol) and triethylamine (1.6 g, 0.0158 mol) in 50 ml EtOAc. The reaction mixture was then stirred under one atmosphere D₂ at room temperature for 24 hr, filtered and the filtrate concentrated to a white powder under reduced pressure. Recrystallization from EtOH (95%) gave white needles, 4.15 g (83%): mp 178-179°; chemical ionization mass spectrum (isobutane) m/e 334 (MH⁺).

5-²H-DL-Tryptophan (39)

To 75 ml of 10% NaOH was added the deuterio formamidopropionate ester 38 (3.9 g, 11.7 mmol). The mixture was refluxed under N₂ atmosphere for 6 hr. After 1 hr all the ester was in solution. After 6 hr, 15.7 ml conc HCl was added followed by 9 ml 2N HCl. A vigorous evolution of gas occurred after addition of conc HCl. The reaction mixture was allowed to cool to room temperature and the pH was adjusted to 6.0. Upon standing overnight at 5°; dull, white crystals were collected and dried to give 1.8 g (75%): dec. 275-280°. Recrystallization from EtOH-H₂O gave white leaflets: mp 288-291° (lit. 289°) (100); chemical ionization mass spectrum (isobutane) m/e 206 (MH⁺).

The first part of the document discusses the importance of maintaining accurate records of all transactions. It emphasizes that every entry should be supported by a valid receipt or invoice. This not only helps in tracking expenses but also ensures compliance with tax regulations. The document further outlines the process of reconciling bank statements with the company's ledger to identify any discrepancies.

In the second section, the author details the various methods used for data collection and analysis. This includes both primary and secondary research techniques. The primary research involves direct observation and interviews with key stakeholders. Secondary research is conducted through a thorough review of industry reports and academic journals. The data collected is then analyzed using statistical software to identify trends and correlations.

The third part of the document focuses on the implementation of the findings. It describes the development of a strategic plan based on the insights gained from the research. This plan includes specific goals, objectives, and a timeline for implementation. The document also discusses the challenges faced during the implementation phase and the strategies used to overcome them.

Finally, the document concludes with a summary of the key findings and recommendations. It highlights the areas where further research is needed and provides a clear path forward for the organization. The author expresses confidence in the effectiveness of the proposed strategies and encourages ongoing monitoring and evaluation.

The following table provides a detailed breakdown of the financial data for the first quarter. It shows the revenue generated from different product lines, the associated costs, and the resulting profit margins. The data indicates a strong performance in the electronics segment, while the services segment shows a slight decline.

Product Line	Revenue	Cost	Profit
Electronics	\$1,200,000	\$750,000	\$450,000
Services	\$800,000	\$600,000	\$200,000
Other	\$300,000	\$200,000	\$100,000
Total	\$2,300,000	\$1,550,000	\$750,000

The analysis of the financial data reveals several key insights. Firstly, the electronics segment remains the primary driver of revenue and profit. However, the services segment has a high potential for growth and should be a focus for future marketing efforts. The 'Other' category, while smaller, shows a consistent profit margin and could be explored further.

The document also includes a section on risk management. It identifies the major risks facing the organization, such as market volatility and supply chain disruptions. Mitigation strategies are proposed, including diversification of suppliers and the implementation of hedging instruments. The author stresses the importance of a proactive risk management approach to ensure the long-term sustainability of the business.

In conclusion, this report provides a comprehensive overview of the company's financial performance and strategic outlook. It offers valuable insights into the strengths and weaknesses of the business and provides a clear roadmap for future success. The author is confident that the proposed strategies will lead to significant growth and profitability in the coming quarters.

N-acetyl-5-²H-DL-tryptophan (40)

To a solution of 5-²H-DL-tryptophan (3.1 g, 15.15 mmol) in 30.5 ml 1.0 N NaOH was added acetic anhydride (1.55 g, 15.15 mmol). The mixture was shaken 10 min, the pH adjusted to 1.0 and a white precipitate collected after cooling overnight. The precipitate was washed with H₂O, dried over P₂O₅ in vacuo and recrystallized from 95% EtOH to give 2.8 g (75%): mp 202-203.5° (lit. 206-7°) (100); chemical ionization mass spectrum (isobutane) m/e 248 (MH⁺).

1-(-)-α-phenylethylamine salt of N-acetyl-5-²H-DL-tryptophan (42)

To a hot solution of 376 mg (5.7 mmol) 85% KOH in 10 ml 95% EtOH was added 2.8 g (11.4 mmol) N-acetyl-5-²H-DL-tryptophan. The mixture was stirred and heated until all of the N-acetyltryptophan was dissolved and 1-(-)-α-phenylethylamine (1.38 g, 11.4 mmol) was added. The solution was allowed to cool to 5° and the resulting crystals were collected by filtration to give 1.5 g (73%): mp 188-191°, $[\alpha]_D^{25} + 20.0^\circ$ (95% EtOH/0.5) (lit. +17.8°) (95).

5-²H-L-tryptophan

The diastereomeric salt 42 (1.1g, 3.0 mmol) was dissolved in 25 ml H₂O and the pH was adjusted to 12.0 with 10% NaOH. The aqueous solution was extracted with benzene, the benzene layer washed with H₂O and the H₂O washings were combined with the aqueous phase. The pH of the aqueous phase was adjusted to 2.0 with 6N HCl and 3

The first part of the paper discusses the importance of the research and the objectives of the study. It then proceeds to a literature review, which identifies the key concepts and theories that will be used in the study. The methodology section describes the research design, the data collection methods, and the data analysis techniques. The results section presents the findings of the study, and the discussion section interprets these findings in the context of the research objectives. The paper concludes with a summary of the main findings and some suggestions for further research.

2. Literature Review: Theoretical Framework and Hypotheses

The theoretical framework of this study is based on the theory of planned behavior (TPB) and the theory of reasoned action (TRA). TPB posits that an individual's intention to perform a behavior is determined by their beliefs about the consequences of that behavior (attitudes toward the behavior) and their beliefs about how others will react to that behavior (normative beliefs). TRA, on the other hand, focuses on the individual's beliefs about the consequences of the behavior (attitudes toward the behavior) and their beliefs about how others will react to that behavior (normative beliefs). The theory of reasoned action (TRA) is a subset of TPB, as it does not include the concept of perceived behavioral control.

In the context of this study, the theory of planned behavior (TPB) is used to explain the factors that influence an individual's intention to engage in a specific behavior. The theory suggests that an individual's intention to perform a behavior is determined by their beliefs about the consequences of that behavior (attitudes toward the behavior) and their beliefs about how others will react to that behavior (normative beliefs). The theory of reasoned action (TRA) is a subset of TPB, as it does not include the concept of perceived behavioral control.

3. Methodology

The study employed a quantitative research design to investigate the relationship between the variables of interest. Data were collected through a survey of a representative sample of individuals. The survey instrument was designed to measure the constructs defined in the theoretical framework. The data were analyzed using statistical techniques to test the hypotheses and to determine the strength of the relationships between the variables.

additional equivalents HCl were added and the solution refluxed for 20 hr under N₂ atmosphere. The reaction mixture was then cooled, treated with charcoal, filtered and the pH adjusted to 5.9. After cooling to 5° the white precipitate was collected and recrystallized from EtOH (abs.) to give shiny white crystals, 279 mg (45%): mp 284-289° (lit. 289° d) (100); $[\alpha]_D^{25}$ -15.8 (H₂O/.5) (standard L-tryptophan $[\alpha]_D^{25}$ -30.8); chemical ionization mass spectrum (isobutane) m/e 205 (MH⁺), 206 (MH⁺) in a ratio of 78:21.

d₄ + d₅-L-tryptophan

A solution of L-tryptophan (4.0 g, 20 mmol) (Aldrich Chemical Co.) in 25 ml D₂O (Aldrich, 99.7+ % atom) and 15 ml 20% DCl in D₂O (Aldrich, 99+ % atom) was refluxed for 18 hr under nitrogen. The solution was then cooled, the pH adjusted to 5.9 and the precipitate collected. One recrystallization from EtOH/H₂O gave fine, white leaflets, 3.8 g (75%): mp 269-272° (lit. 289° d) (100), $[\alpha]_D^{25} = -31.0^\circ$ (100); chemical ionization mass spectrum (isobutane) 34.2% m/e 209 (MH⁺ + 4), 65.8% m/e 210 (MH⁺ +5).

d₅-L-tryptophan

A solution 3.0 g L-tryptophan in 15 ml D₂O (99.7 + % atom) was stirred for 1 hr at room temperature, the solvent was removed in vacuo and 10 ml d₁-trifluoroacetic acid (Stahler Isotope, 99% atom) was added. The resulting solution was stirred 5 hr under nitrogen in the dark. The solvent was then removed and this procedure repeated 4 times. After removing the last traces of solvent in vacuo, the residue was recrystallized from EtOH/H₂O to give fine, white leaflets,

1.95 g (65%): mp 269-272° (lit. 289° d) (100), $[\alpha]_D^{20} = -31.0^\circ$ (lit. $[\alpha]_D^{20} = -31.5^\circ$) (100). NMR (d_1 -TFAA) δ 3.5 (d, 2H, β -H), 4.5 (t, 1H, α -H), 6.9-7.6 (m, .29H, 2-H, 4-H, 5-H, 6-H, 7-H); chemical ionization mass spectrum (isobutane) m/e 210 ($MH^+ + 5$).

Ethyl 3-Indolyglyoxylate (48)

A solution of indol (3.7 g, 0.032 mol) in 50 ml anhydrous Et₂O was cooled to 0-5° and stirred while oxalyl chloride (4.6 g, 0.036 mol) was added dropwise over a period of 20 min. Cooling and stirring was continued for 1 hr after which 60 ml absolute EtOH and 5 ml Et₃N were added and the reaction mixture refluxed for 30 min. After cooling, the precipitate was collected and recrystallized from EtOH-H₂O to give fine white needles: 5.45 g (80%); mp 183-185°, (lit. mp 186°) (106).

d₄-Tryptophol (49)

A suspension of ethyl-3-indolyglyoxylate (5.0 g, 0.023 mol) and lithium aluminum deuteride (3.36 g, .08 mol) in 100 ml dry THF was refluxed for 2 hr under N₂ atmosphere. The excess hydride was decomposed by addition of 10 ml H₂O. The resulting precipitate was filtered, washed with THF, the combined filtrates dried over Na₂SO₄ and the solvent removed in vacuo to give a green oil. Upon standing and cooling, white crystals formed, which were collected and recrystallized from benzene-petroleum ether (65-110°) to give white platelets, analytically pure: mp 57.5-58.5° (lit. mp 58-9°) (106); chemical ionization mass spectrum (isobutane) m/e 166 (MH⁺). Anal. calc for C₁₀H₇D₄ NO: C, 72.7; H, 9.1; N, 8.5. Found: C, 72.93; H, 8.88; N, 8.64.

The first part of the course covers the basic concepts of algebra, including the properties of real numbers, the operations of addition, subtraction, multiplication, and division, and the concept of exponents. The second part of the course covers the basic concepts of geometry, including the properties of lines, angles, triangles, and quadrilaterals. The third part of the course covers the basic concepts of trigonometry, including the properties of sine, cosine, and tangent functions, and the concept of the unit circle. The fourth part of the course covers the basic concepts of calculus, including the concept of limits, the derivative, and the integral.

The course is designed to provide a solid foundation in mathematics for students who are interested in pursuing a career in science, engineering, or business. The course is also suitable for students who are preparing for the SAT or ACT exams.

The course is divided into four main sections: Algebra, Geometry, Trigonometry, and Calculus. Each section is further divided into several chapters, each covering a specific topic. The course is designed to be self-paced, allowing students to learn at their own speed. The course is also available in a condensed format, allowing students to complete the course in a shorter period of time. The course is taught by experienced instructors who are passionate about teaching mathematics. The course is also supported by a variety of resources, including textbooks, online materials, and practice problems. The course is designed to be both challenging and rewarding, providing students with a deep understanding of the fundamental concepts of mathematics. The course is also designed to be accessible to students of all backgrounds and abilities. The course is a great way to learn mathematics and to develop the skills and knowledge needed for success in many careers.

d₄-5-Benzyloxytryptophol (50)

To a solution of 5-benzyloxyindole (5.0 g, 22.4 mmol) in 50 ml ether at 0-5° was added, dropwise over 15 min, oxalyl chloride (3.3 g, 26 mmol). The reaction mixture was stirred 1 hr and filtered to give a red powder which was dried in vacuo to give 26 g (38%). The crude 5-benzyloxyindolygly oxalyl chloride (47) was added to a suspension of lithium aluminum deuteride (1.43 g, 34.1 mmol) in 40 ml dry THF and refluxed 2 hr under N₂ atmosphere. The reaction mixture was decomposed with 10 ml H₂O, the precipitate filtered and washed with THF and the combined THF fractions were dried over Na₂SO₄ and concentrated to an oil in vacuo. Upon standing at 5° the oil solidified and three recrystallizations from benzene/petroleum ether (65-110°) gave 875 mg (38%): mp 92-95° (lit. 93-95°) (*107*); chemical ionization mass spectrum (isobutane) m/e 272 (MH⁺).

d₄-5-Hydroxytryptophol (51)

A solution of d₄-5-benzyloxytryptophol (850 mg, 3.1 mmol) in 25 ml MeOH was treated at s.t.p. with 10% Pd/BaSO₄ (500 mg) under H₂ atmosphere. After 3 hr the hydrogenolysis was complete and the reaction mixture was filtered and the solvent removed in vacuo. The residue was dissolved in EtOAc, treated with charcoal and cooled to 5°. Petroleum ether (65-110°) was added until the solution turned cloudy, the solution was allowed to stand at 5° and fine white crystals were collected to give 110 mg (20%): mp 107-109 5° (lit. 105-107°) (*107*); chemical ionization mass spectrum (isobutane) m/e 182 (MH⁺). Anal. calc. for C₁₀H₇D₄NO₂: C, 66.3; H, 8.3; N, 7.7. Found: C, 66.43; H, 8.06; N, 7.52.

1. The first step in the process of identifying a problem is to recognize that a problem exists. This is often done by comparing current performance to a desired state or goal. Once a problem is identified, the next step is to define the problem more precisely. This involves identifying the causes of the problem and the scope of the problem. The third step is to generate potential solutions. This is often done by brainstorming or using a structured problem-solving process. The fourth step is to evaluate the potential solutions. This involves comparing the solutions to the problem and to each other. The fifth step is to select a solution. This is often done by choosing the solution that is most likely to be successful. The sixth step is to implement the solution. This involves putting the solution into action. The seventh step is to evaluate the results. This involves comparing the results to the desired state or goal. If the results are not satisfactory, the process may need to be repeated.

Problem Solving Process

1. The first step in the process of identifying a problem is to recognize that a problem exists. This is often done by comparing current performance to a desired state or goal. Once a problem is identified, the next step is to define the problem more precisely. This involves identifying the causes of the problem and the scope of the problem. The third step is to generate potential solutions. This is often done by brainstorming or using a structured problem-solving process. The fourth step is to evaluate the potential solutions. This involves comparing the solutions to the problem and to each other. The fifth step is to select a solution. This is often done by choosing the solution that is most likely to be successful. The sixth step is to implement the solution. This involves putting the solution into action. The seventh step is to evaluate the results. This involves comparing the results to the desired state or goal. If the results are not satisfactory, the process may need to be repeated.

Part III

Assay for Urinary Metabolites of Tryptophan

A. Chemical Ionization Mass Spectrometry

Chemical Ionization Mass Spectrometry

The main advantage of chemical ionization mass spectrometry (C.I.M.S.) over electron impact mass spectrometry (E.I.M.S.) is the decreased fragmentation of the molecule and the appearance of a parent peak for even the most unstable compounds (31,108). The ability to see the parent peak of compounds of biologic interest rests as an important step in the identification of a myriad of metabolites.

Chemical ionization affords parent peaks of $m/e = MH^+$ as protonation is the means by which the molecule is imparted a positive charge in order to be accelerated. This is contrasted with the removal of an electron as in E.I.M.S. Protonation of a compound involves less energy than ionization by E.I. and thus less destruction (fragmentation) of the molecule. Though E.I.M.S. employs an electron beam of 70 ev. and C.I.M.S. uses 650-675 ev., the higher energy involved in C.I. is needed for the formation of the protonating species, i.e., CH_5^+ in methane C.I., not for the protonation of the compound. Protonation of the compound is a result of molecular collisions forming an MH^+ molecule of low energy. Formation of an M^+ molecule by E.I. results in the molecule existing in an excited state that can and will fragment. The MH^+ ion is very stable compared to the odd-electron radical ion derived from the molecule by electron impact. What fragmentation that does exist in C.I. is usually simple as Milne, Axenrod and Fales have reported on the methane C.I. of alkaloids and amino acids (31,109).

Isobutane chemical ionization involves even lower energy than methane C.I. as the protonating species, the t-butyl cation ($C_4H_9^+$) is

a milder Bronsted acid than CH_5^+ . Thus, isobutane C.I. affords spectra consisting of only a parent peak, MH^+ and very little fragmentation, usually of intensity of less than 5%. In cases where there is some fragmentation of the MH^+ ion, it usually follows the familiar rules of acid catalyzed reactions.

Isobutane chemical ionization is very close to being monoionic at pressures of 0.5-1.0 torr. I conducted isobutane C.I.M.S. at pressure approximately 0.7 torr which results in an ion distribution of m/e 57, $\text{t-C}_4\text{H}_9^+$, 91.8%; m/e 43, C_3H_7^+ , 0.3%; m/e 39, C_3H_3^+ , 3.2%. The relative intensities of isobutane ions varies with the pressure and temperature. Figures 26a,b,c,d show E.I. and C.I. mass spectrum of IAA and 5-HIAA and are presented here for comparison.

Recently colleagues and myself have been experimenting with various compounds to determine their suitability as a reagent (Bronsted acid) for chemical ionization. Those that have been examined are H_2 , D_2 , methanol, dioxane and isopropyl ether. The optimum reagent would be a compound that is a very mild Bronsted acid so as to afford essentially peaks (MH^+) with very little fragmentation, i.e., a compound that would render essentially a mono-ionic species (as isobutane is 91% t-butyl cation) and have the physical properties that render it suitable for use in the MS-9 spectrometer at the pressures used. Methanol is interesting in that at source pressures of 0.7-0.8 torr, (200°), the C.I. spectrum consists of m/e = 33 (MH^+), 13%; m/e = 65 (dimer ($2\text{M}+\text{H}$) $^+$), 69%; m/e = 97 (trimer), 7%; m/e = 47, 6%; and m/e = 79, 4%. Dioxane shows a C.I.

spectrum consisting of $m/e = 89$, (MH^+) 45%; $m/e = 177$ (dimer ($2M+H$)⁺), 45%; and $m/e = 88$ and 87 . Isopropyl ether gives a very clear C.I. spectrum consisting of $m/e = 103$ (MH^+), 64%; $m/e = 87$, 13%; and $m/e = 61$ ($C_3H_9O^+$), 16%.

Isopropyl ether C.I. was used to analyze the urine of a Type IV carcinoid patient. (See section on patient assays.)

Part III

B. Assay Technique

C.I.M.S. Assay

The demonstration of chemical ionization mass spectrometry utility by Fales and Axenrod (31) convinced me that C.I.M.S. may be useful in identifying metabolites excreted in the urine. The gentleness of chemical ionization that affords parent peaks (MH^+) of unstable molecules was useful in determining the presence of several indolic metabolites. Figure 26 show chemical ionization mass spectra of two indolic metabolites compared with their electron impact mass spectra. In each case, the parent peak is much greater in the C.I. spectrum than in the E.I. spectrum. Table 8 shows the total ion current measured for 5-HIAA and IAA.

Table 8

Total ion current of a chemical ionization mass spectrum (isobutane) of 5-HIAA and IAA

<u>5-HIAA</u>				<u>IAA</u>			
<u>Mass</u>	<u>molecule</u>	<u>total ion</u>	<u>% ion</u>	<u>mass</u>	<u>molecule</u>	<u>total ion</u>	<u>% ion</u>
248	M + 57	23	2.4	232	M + 57	24	3.8
234	M + 43	15	1.5	218	M + 43	11	1.7
230	M + 39	19	2.0	214	M + 39	10	1.6
193	$^{13}CMH^+$	90	9.3	177	$^{13}CMH^+$	51	8.0
192	MH^+	700	72.7	176	MH^+	500	78.9
191	M^+	33	3.4	175	M^+	21	3.3
148	MH^+-CO_2	31	3.2	130	$MH^+-H_2CO_2$	17	2.7
146	$MH^+-H_2CO_2$	52	5.4				

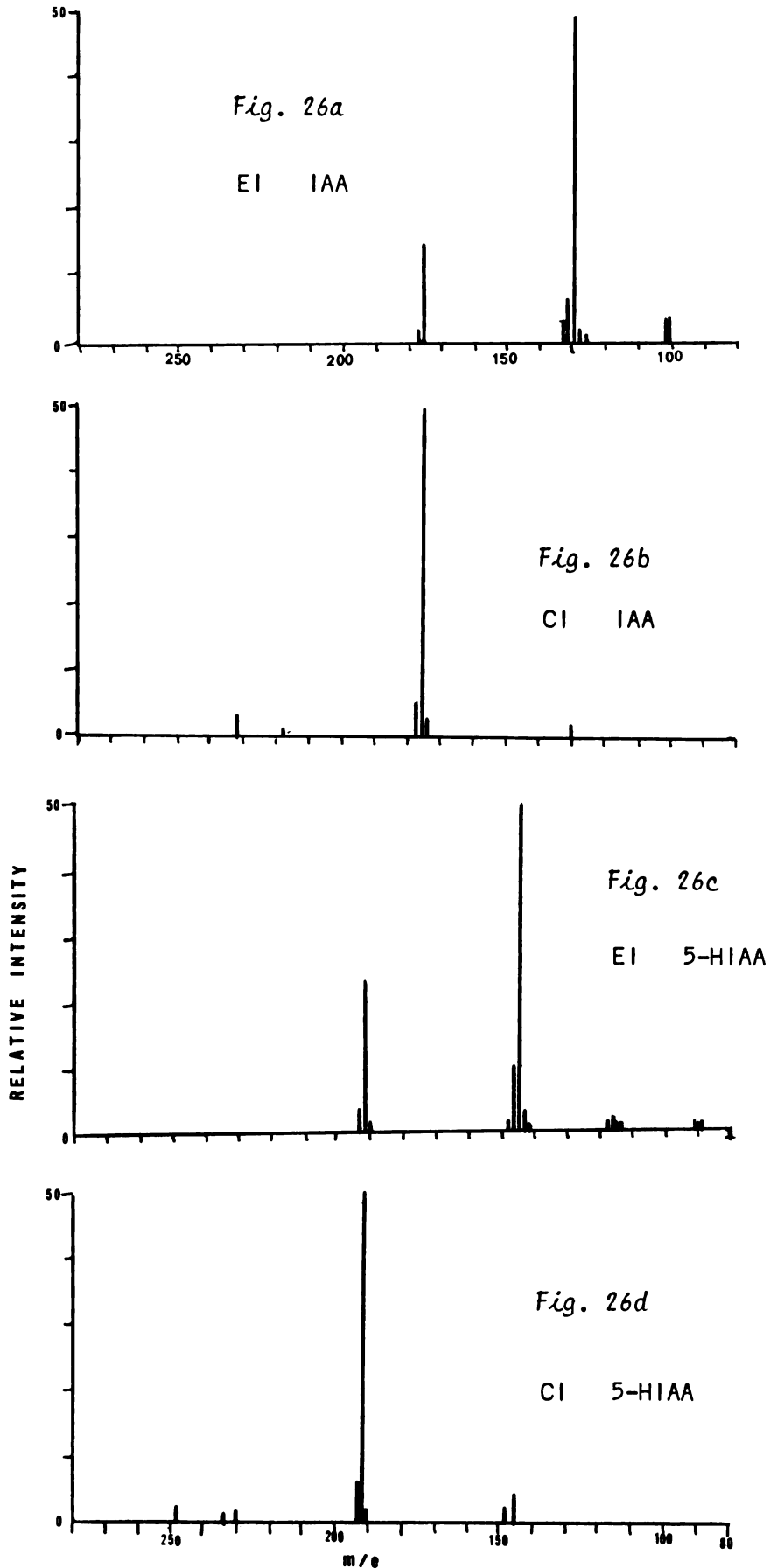


Fig. 26a,b,c,d Comparison of Electron Impact (EI) and Chemical Ionization (CI) mass spectra of IAA and 5-HIAA.



C.I. does produce some fragmentation and some higher massed compounds when a molecule combines with the reactant gas, in this case isobutane. A mass at $M + 57$ results from combination with the tertiary butyl cation, $C_4H_9^+$; the mass at $M + 43$ results from combination with the isopropyl cation, $C_3H_7^+$; the mass at $M + 39$ results from combination with the $C_3H_3^+$ ion. Fragmentation of acids usually consists of loss of CO_2 and H_2CO_2 from the molecular ion MH^+ .

Thus, with C.I.M.S. producing a parent peak (plus ^{13}C peak) of 82% of the total ion current for 5-HIAA and almost 87% of the total ion current for IAA, it was thought that high resolution continuous ion monitoring of the two parent peaks of these two compounds would produce a ratio of the relative amounts of these compounds present without interference from other compounds. The subsequent addition of a known amount of one of the compounds and re-determining the ratio of the amounts present would give after computation, the amounts of the two compounds in the original mixture. I hoped to use this method to quantify several indolic acids.

Using this procedure, I attempted to determine the ratio of 5-HIAA to IAA present in solutions of known amounts. Table 9 shows the results of C.I.M.S. assay and gas chromatograph assay using solutions of IAA (Sigma Chem. Co.) and 5-HIAA (Sigma Chem Co.) in methanol (Spec. grade, Aldrich Chem. Co.).

Table 9

Chemical ionization mass spectrometric (isobutane) and gas chromatographic ratios of known mixtures of IAA and 5-HIAA

Ratio IAA/5-HIAA

<u>Calculated</u>	<u>Observed C.I.M.S.</u>	<u>Observed G.C.</u>
1:1	5:1	1.53: 1
1:1	4:1	
2:1	10:1	3.14: 1
2:1	11:1	
5:1	35:1	8.6: 1
5:1	32:1	
10:1	91:1	14.5: 1
10:1	1:1	
1:10	1:1	1:4.6
1:10	1:12	
1:10	1:3	
1:5	1:1.6	1:3.2
1:2	3:1	1:1
1:2	1:1	

The procedure was repeated again with freshly prepared solutions of fresh standards. Table 10 shows the results of C.I.M.S. and G.C. assay. Figures 27, 28 and 29 show graphs of IAA/5-HIAA ratios determined by C.I.M.S. continuous ion monitoring.

Fig. 27 CIMS continuous ion monitoring of m/e 176 (IAA) and m/e 192 (5-HIAA) of a standard solution of IAA/5-HIAA ratio 1:10.

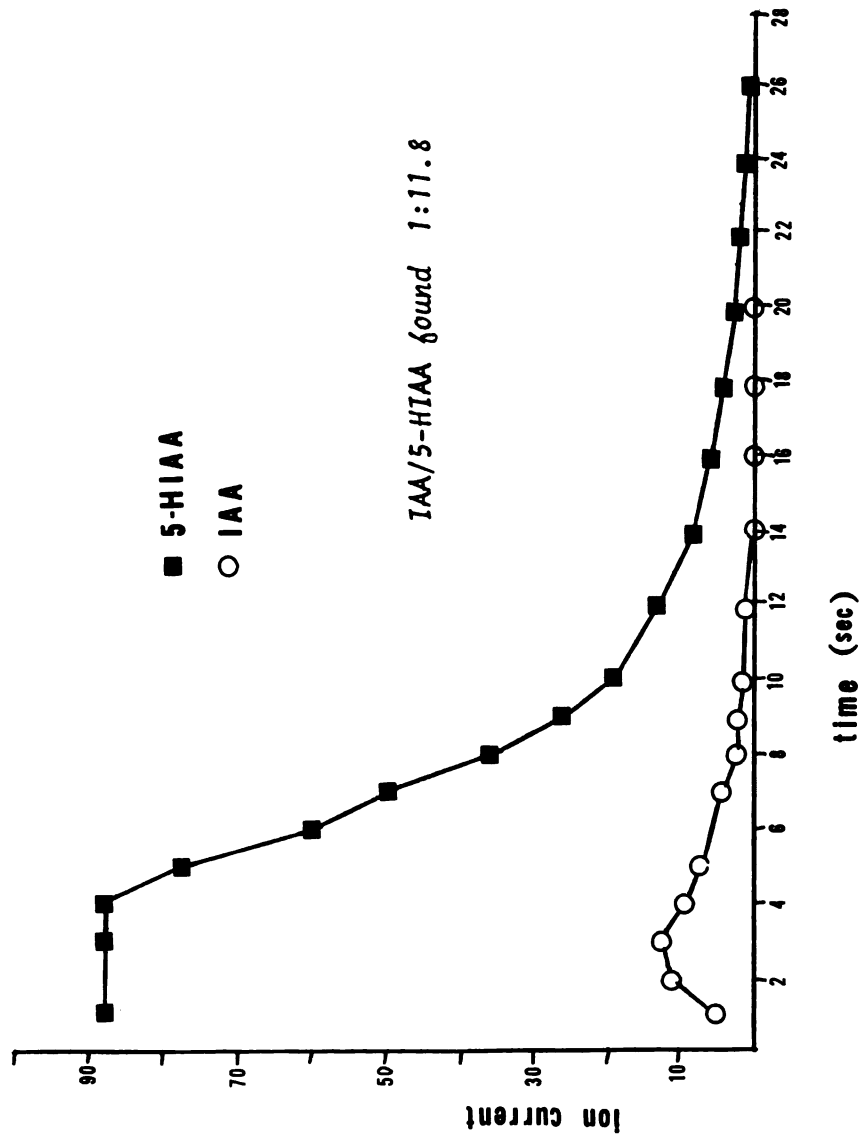
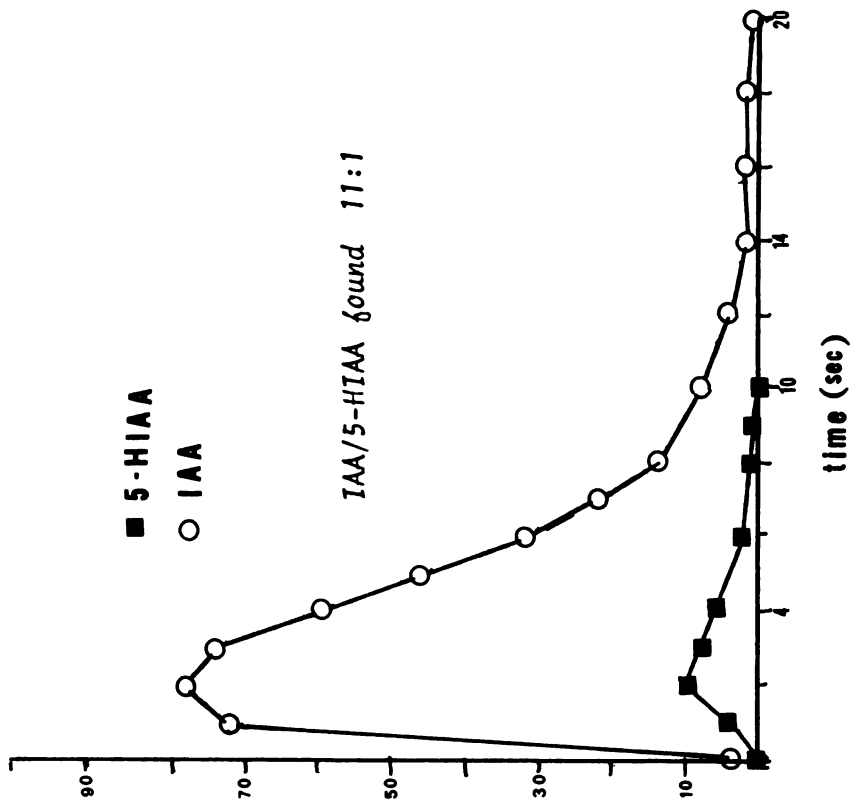


Fig. 28 CIMS continuous ion monitoring of m/e 176 (IAA) and m/e 192 (5-HIAA) of a standard solution of IAA/5-HIAA ratio 10:1.



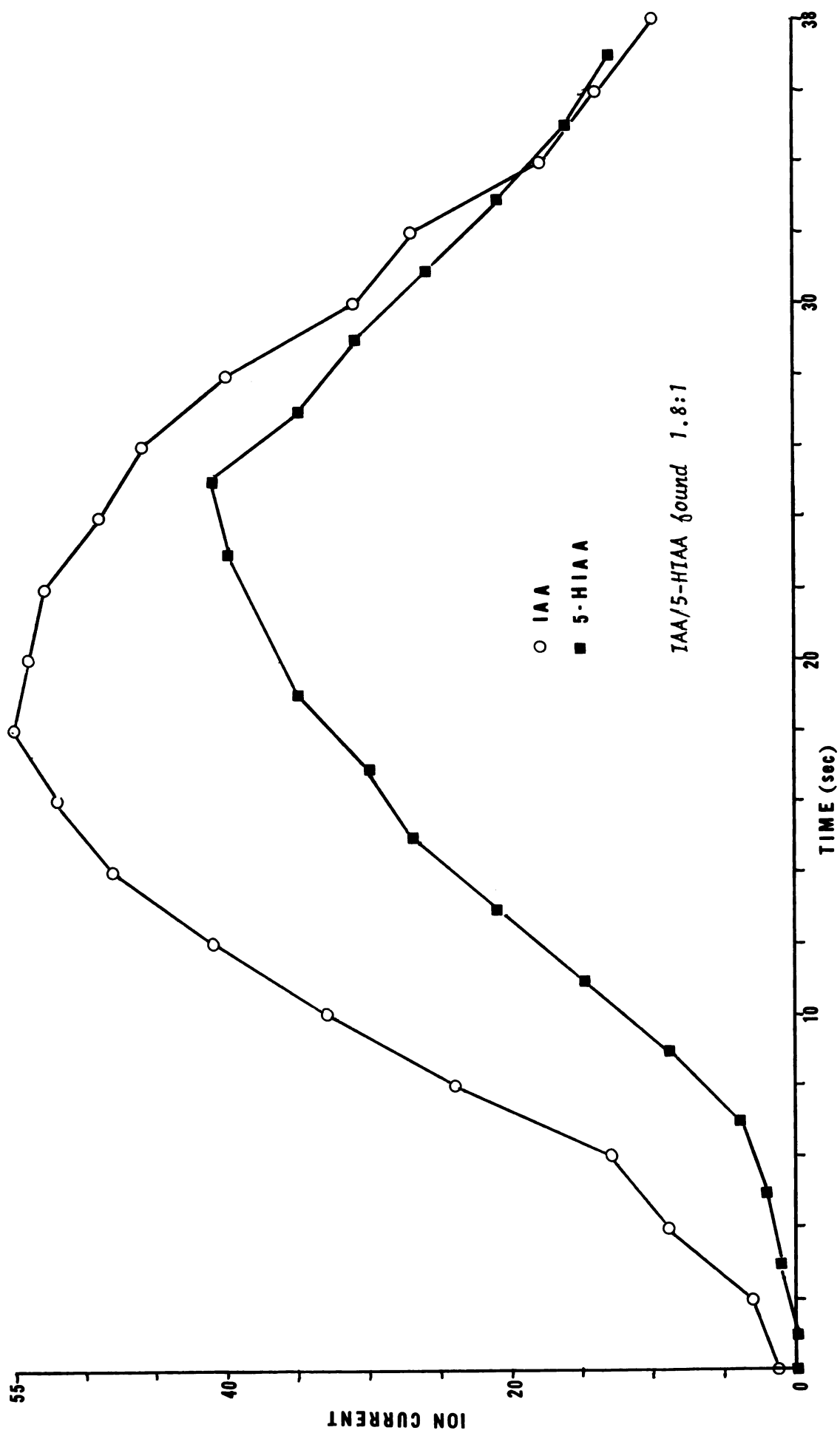


Fig. 29 CIMS continuous ion monitoring of m/e 176 (IAA) and m/e 192 (5-HIAA) of a standard solution of IAA/5-HIAA ratio 1:1.



Table 10

Chemical ionization mass spectrometry (isobutane) and gas chromatographic ratios of known mixtures of IAA and 5-HIAA.

Ratio IAA: 5-HIAA

<u>Calculated</u>	<u>Observed C.I.M.S.</u>	<u>Observed G.C.</u>
1:1	1.67:1	1.8:1
2:1	3.3:1	2.5:1
1:2	1:1	1:1

Due to the different volitilities of the two compounds, however, continuous monitoring of the ion current would have to be performed until both compounds were completely evaporated from the probe. I found that the greater the difference in volitility, the greater the error in the ratio found. I decided to use derivatives of these compounds to increase their volitility and make measurement more accurate.

Derivatization of compounds for mass spectral analysis is a common procedure. Replacement of active hydrogens by trimethylsilyl, pentafluoropropional (110) or heptafluorobutyryl groups (111) and the use of methyl esters and methyl ethers are currently the most common methods in use. Bertilsson and Palmer (111) describe a mass fragmentography assay for IAA using the heptafluorobutyryl methyl ester derivative.

1. The first part of the document discusses the importance of maintaining accurate records of all transactions.

2. It is essential to ensure that all entries are supported by appropriate documentation and receipts.

3. Regular audits should be conducted to verify the accuracy of the records and identify any discrepancies.

4. The second part of the document outlines the procedures for handling disputes and resolving conflicts.

5. It is important to establish clear communication channels and protocols for addressing any issues that arise.

6. The third part of the document provides a detailed overview of the financial statements and reports.

7. These reports should be prepared on a regular basis and presented to the relevant stakeholders.

8. The fourth part of the document discusses the role of the management team in overseeing the organization's operations.

9. It is crucial for management to stay informed about the company's performance and make strategic decisions.

10. The fifth part of the document concludes with a summary of the key findings and recommendations.

11. It is hoped that this report will provide valuable insights and guidance for the organization's future success.

12. The sixth part of the document provides a detailed overview of the financial statements and reports.

13. These reports should be prepared on a regular basis and presented to the relevant stakeholders.

14. The seventh part of the document discusses the role of the management team in overseeing the organization's operations.

15. It is crucial for management to stay informed about the company's performance and make strategic decisions.

I decided to prepare the methyl ester derivatives of acids and the methyl ether derivatives of phenolic compounds by reaction with diazomethane. I found this method to be quick and efficient. The methyl esters are produced essentially instantaneously upon contact with diazomethane while the methyl ethers of phenols were treated twice to effect quantitative methylation. In addition to increased volatility, the methylated compounds fragmented much less under chemical ionization conditions due to the lower temperatures necessary to volatilize the derivative. The C.I.M.S. of indoleacetic acid methyl ester (IAA·ME) was nearly 99% m/e 192 (MH^+) and that of 5-hydroxyindoleacetic acid methyl ester methyl ether (5-HIAA·ME·ME) nearly 98% m/e 220 (MH^+) (figs. 30,31).

Solutions of known amounts of IAA and 5-HIAA determined on a Cahn balance to ± 0.01 mg were treated with ethereal diazomethane, evaporated to dryness and dissolved in a known volume of solvent. Standard mixtures of IAA ME and 5-HIAA·ME·ME were prepared by adding known volumes of the above solution using a microliter syringe. The ratios of IAA and 5-HIAA present were then determined by continuous monitoring of m/e 192 and m/e 220. The results are tabulated in table 11 and plotted in figure 32.

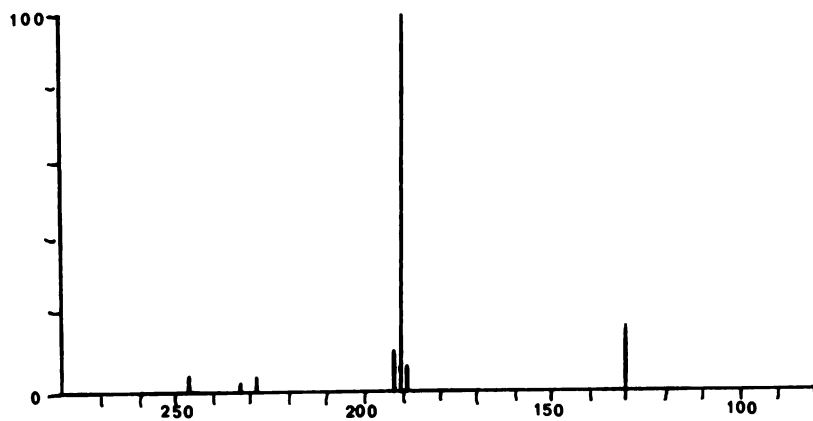


Fig. 30 CIMS of IAA methyl ester (mw 189) at 190°C.

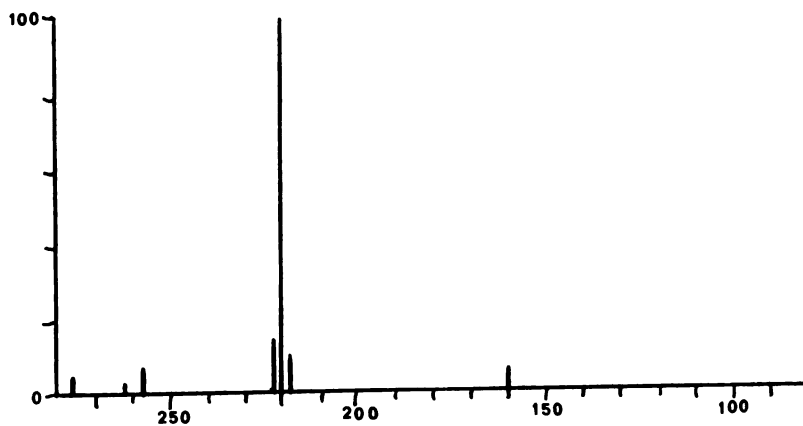


Fig. 31 CIMS of 5-HIAA methyl ester, methyl ether (mw 219) at 190°C.

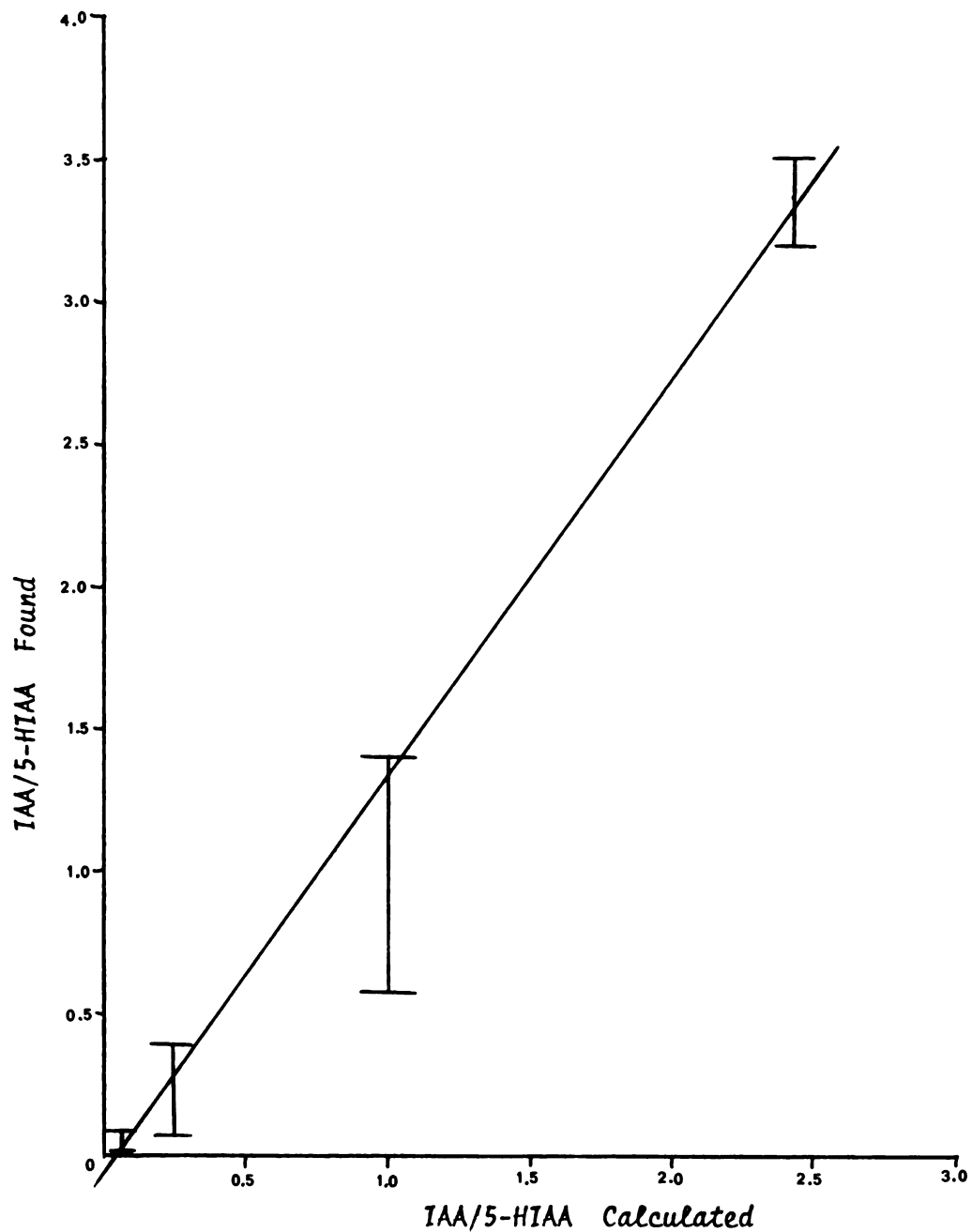


Fig. 32

IAA/5-HIAA ratio found vs calculated. Ratio found determined by CIMS continuous ion monitoring of m/e 190 (IAA methyl ester) and m/e 220 (5-HIAA methyl ester-methyl ether).

11
12
13

14

15

16
17
18

Table 11

Chemical ionization mass spectrometry (isobutane) ratios of IAA·ME and 5-HIAA·ME·ME obtained by continuous ion monitoring of the par peaks (190 and 220).

<u>mixture no.</u>	<u>calc.</u>	<u>Ratio IAA/5-HIAA</u>						<u>ave</u>
		<u>observed</u> <u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	
1	0.25	0.30	0.14	0.18	0.39			0.25
2	2.42	3.5	3.2	3.2				3.3
3	0.06	0.04	0.03	0.05	0.08			0.05
4	1.0	1.0	1.0	0.6	0.58	1.2	1.4	.96

These results showed a wide variation in the ratios obtained. The average of the ratios for mixtures one, three and four were very close to the actual ratio added. Mixture two was off by a factor of 1.4, unacceptable for quantitative work. Following the assay procedure to completion, known amounts of IAA·ME or 5-HIAA·ME·ME were added to the above mixtures. The resultant values for amounts present differed from the actual amounts by 35 per cent. This large error and the obvious inability to obtain consistent ratios led me to abandon this assay and approach the problem of quantification by way of an assay employing an internal standard. Thus, I embarked on a program to synthesize deuterium labeled standards which was described in a previous section and to employ these compounds in a C.I.M.S. assay. A description of the assay follows.

the fact that the CO_2 concentration in the atmosphere is increasing rapidly, and that the concentration of CO_2 in the atmosphere is a major factor in determining the rate of global warming.

The following table shows the concentration of CO_2 in the atmosphere in parts per million (ppm) from 1750 to 2000. The concentration of CO_2 in the atmosphere has increased from about 280 ppm in 1750 to about 370 ppm in 2000.

Year	Concentration (ppm)
1750	280
1800	280
1850	285
1900	295
1950	310
2000	370

Source: National Oceanic and Atmospheric Administration (NOAA)

The following table shows the concentration of CO_2 in the atmosphere in parts per million (ppm) from 1750 to 2000. The concentration of CO_2 in the atmosphere has increased from about 280 ppm in 1750 to about 370 ppm in 2000.

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Source: National Oceanic and Atmospheric Administration (NOAA)

Procedure for Internal Standard C.I.M.S. Assay

A patient's 24 hr urine is collected and stored frozen without preservative until assayed. The frozen urines were thawed, the total volume and pH recorded and a 100 ml. aliquot removed for assay. Known amounts of deuterium labeled standards were added to this aliquot in amounts equivalent to 500-100 $\mu\text{g}/24$ hr urine. The urinary pH was adjusted to 7.0 by aqueous base and extracted with three, 35 ml portions of ethyl acetate. The ethyl acetate fractions were combined, dried over anhydrous sodium sulphate and concentrated to dryness in a stream of nitrogen. This fraction containing neutral compounds, phenols and weak bases was then submitted for C.I.M.S. analysis. The remaining urine fraction was brought to pH 1.8 by addition of 6.0 N HCl, saturated with NaCl and extracted with three 35 ml portions of ethyl acetate. These ethyl acetate fractions were combined, dried over anhydrous sodium sulphate and concentrated to dryness. This fraction containing organic acids was then submitted to C.I.M.S., t. l.c. and g.l.p.c. analysis.

1. The first step in the process of identifying a problem is to recognize that a problem exists. This is often done by comparing current performance with a desired state or goal. For example, a manager might notice that sales are declining or that customer satisfaction is low. Once a problem is identified, the next step is to define it more precisely. This involves determining the scope of the problem, its causes, and its effects. For instance, a manager might define a sales decline as a 10% drop in revenue over the last quarter, caused by a decrease in the number of new customers and a loss of existing customers. The third step is to analyze the problem. This involves gathering data, identifying patterns, and testing hypotheses. For example, a manager might analyze sales data to see if there is a seasonal trend or if the decline is more pronounced in certain regions. The fourth step is to generate potential solutions. This is often done by brainstorming or using problem-solving techniques. For example, a manager might generate solutions such as increasing marketing efforts, improving customer service, or offering discounts. The fifth step is to evaluate the potential solutions. This involves comparing the solutions against criteria such as cost, feasibility, and potential impact. For example, a manager might evaluate the cost of increasing marketing efforts and the potential impact on sales. The sixth step is to select a solution. This is often done by choosing the solution that is most likely to solve the problem and is most feasible. For example, a manager might select the solution of increasing marketing efforts. The seventh step is to implement the solution. This involves putting the solution into action. For example, a manager might implement the solution by increasing the budget for marketing. The eighth step is to monitor the results. This involves tracking the performance of the solution over time. For example, a manager might monitor sales data to see if there is an improvement. The ninth step is to evaluate the results. This involves comparing the results against the original goal. For example, a manager might evaluate the results by comparing sales data to the original goal of a 10% increase in revenue. The tenth step is to adjust the solution if necessary. This involves making changes to the solution if it is not working. For example, a manager might adjust the solution by increasing the budget for marketing further.

Part III

C. Assay Standardization

Assay Standardization

Tryptophol and 5-Hydroxytryptophol

Standard curves for quantifying tryptophol (TOH) and 5-hydroxytryptophol (5-HTOH) were obtained by adding known amounts of TOH and 5-HTOH and their d_4 analogs to aqueous buffer or urine and subsequently extracting with ethyl acetate after adjusting the pH to 7.0. C.I.M.S. analysis of the extract enabled quantitation by measuring the peak ratio of MH^+ 162/166 for TOH and MH^+ 178/182 for 5-HTOH. The ratio times the amount of d_4 compound added gave the amount of TOH or 5-HTOH found and this was plotted against the calculated amount that was used. Solutions of TOH and 5-HTOH and their d_4 analogs consisted of known amounts weighted to $0.01 \text{ mg} \pm 0.0005 \text{ mg}$ on a Cahn Electrobalance and dissolved in 0.2M phosphate buffer, pH 7.4 and had the following concentrations:

tryptophol	0.87 $\mu\text{g}/\mu\text{l}$
d_4 -tryptophol	0.51 $\mu\text{g}/\mu\text{l}$
5-hydroxytryptophol	0.79 $\mu\text{g}/\mu\text{l}$
d_4 -5-hydroxytryptophol	0.71 $\mu\text{g}/\mu\text{l}$

Microliter quantities between 100 and 300 μl were pipetted using a Rainin adjustable microliter pipet accurate to $\pm 0.1 \mu\text{l}$. Quantities between 10 and 20 μl were pipetted using a Rainin pipet accurate to $\pm 0.01 \mu\text{l}$.

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2. The first step in the process of identifying a problem is to recognize that a problem exists. This is often done by comparing current performance with a desired state or goal. For example, a manager might notice that sales are declining or that customer satisfaction is low. Once a problem is identified, the next step is to define it more precisely. This involves determining the scope of the problem, its causes, and its effects. For instance, a manager might define a problem as "a 10% decrease in sales over the last quarter, primarily due to a loss of market share in the competitive market." The third step is to analyze the problem. This involves gathering data, identifying key factors, and determining the underlying causes. For example, a manager might analyze sales data to identify trends, compare performance with competitors, and identify areas where the company is losing market share. The fourth step is to generate potential solutions. This involves brainstorming ideas and evaluating them based on their feasibility, effectiveness, and cost. For instance, a manager might generate several potential solutions, such as increasing marketing efforts, improving product quality, or offering discounts to customers. The fifth step is to select a solution. This involves choosing the most promising solution based on the analysis and the evaluation of potential solutions. For example, a manager might select a solution that involves increasing marketing efforts and improving product quality. The sixth step is to implement the solution. This involves putting the chosen solution into action and monitoring its progress. For instance, a manager might implement a marketing campaign and track sales and customer satisfaction over time. The seventh step is to evaluate the results. This involves comparing the actual results with the desired state and determining whether the problem has been solved. For example, a manager might evaluate sales and customer satisfaction data to determine if the marketing campaign and product improvements have led to an increase in sales and customer satisfaction. If the problem has not been solved, the manager may need to re-evaluate the solution and try a different approach.

The standard curves for TOH and 5-HTOH can be seen in figures 33 and 34. The assay for tryptophol extracted out of urine and buffer is fairly consistent over a range of from 40 μg to 280 μg . This would be equivalent to 0.6 to 4.2 mg/24 hr urine (using 1500 ml as an average 24 hr. urine for computation purposes). The relationship between μg found and μg calculated is linear which allows for extrapolation when higher amounts are used. The usual method of obtaining a standard curve when using an internal standard is keeping the amount of internal standard added constant, varying the known amount added of compound under study and then plotting the ratio of peak height vs amount of compound under study added (110,111,112). I found this approach unsatisfactory in that when the peak heights are vastly different, a large amount of error is involved in their measurement and ratio determination. Accuracy is greatest when the ratio is closest to 2:1 or 1:1. Thus, when analyzing an unknown sample, an approximate value will be obtained and the sample will then be analyzed again but with a known amount of d_4 compound added to approximate 1:1 or 2:1 ratio.

The standard curve for 5-HTOH is linear over a range of 6-240 μg . This would be equivalent to 0.09 - 3.6 mg/24 hr urine. This value as well as the value for tryptophol is not the limit of detection. The chemical ionization mass spectrometer is fitted with an ion current monitor that can monitor and record two peaks simultaneously. The lower limit of detection is around 10^{-12} grams.

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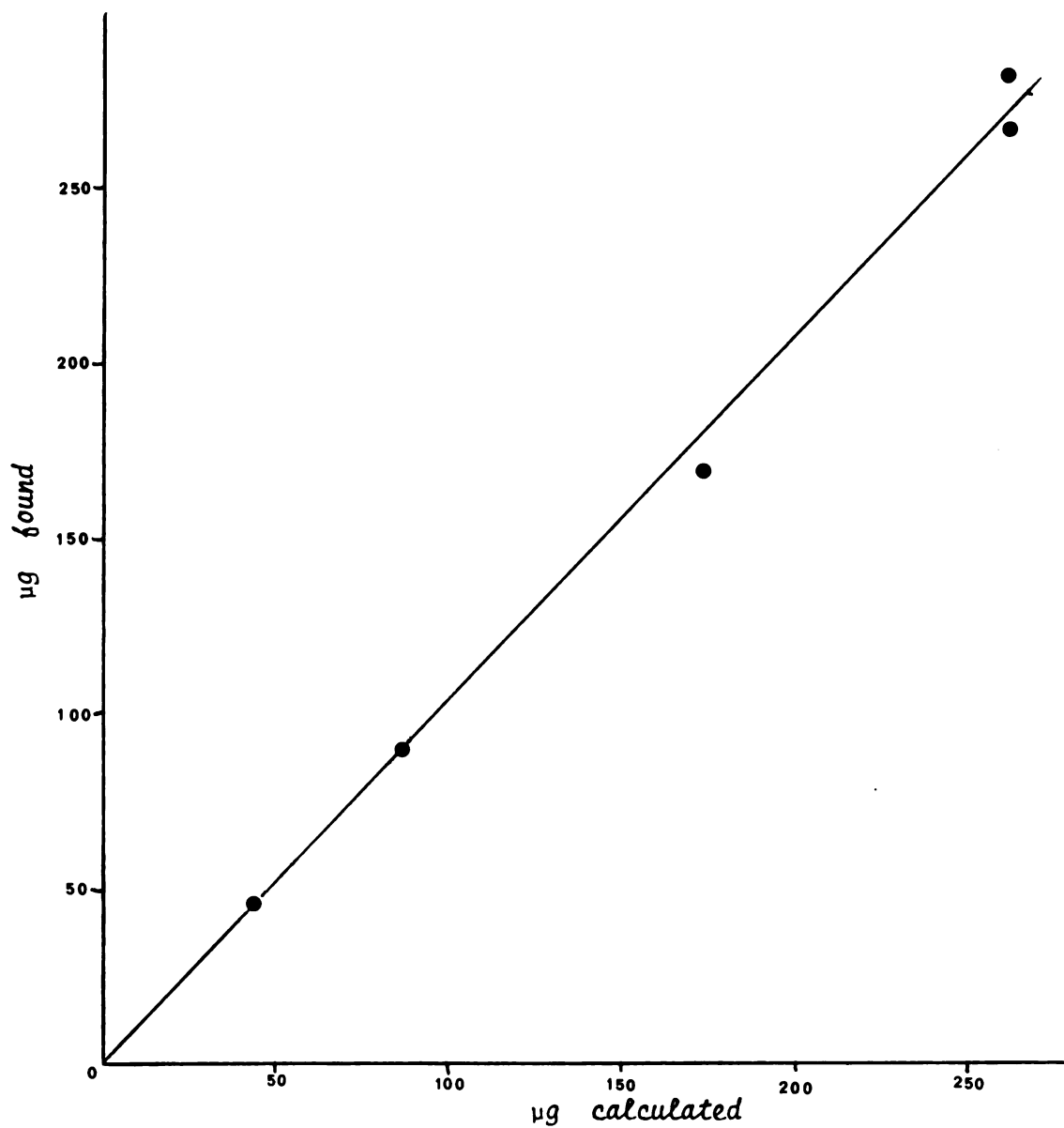


Fig. 33 Tryptophol standard curve determined by CIMS assay.

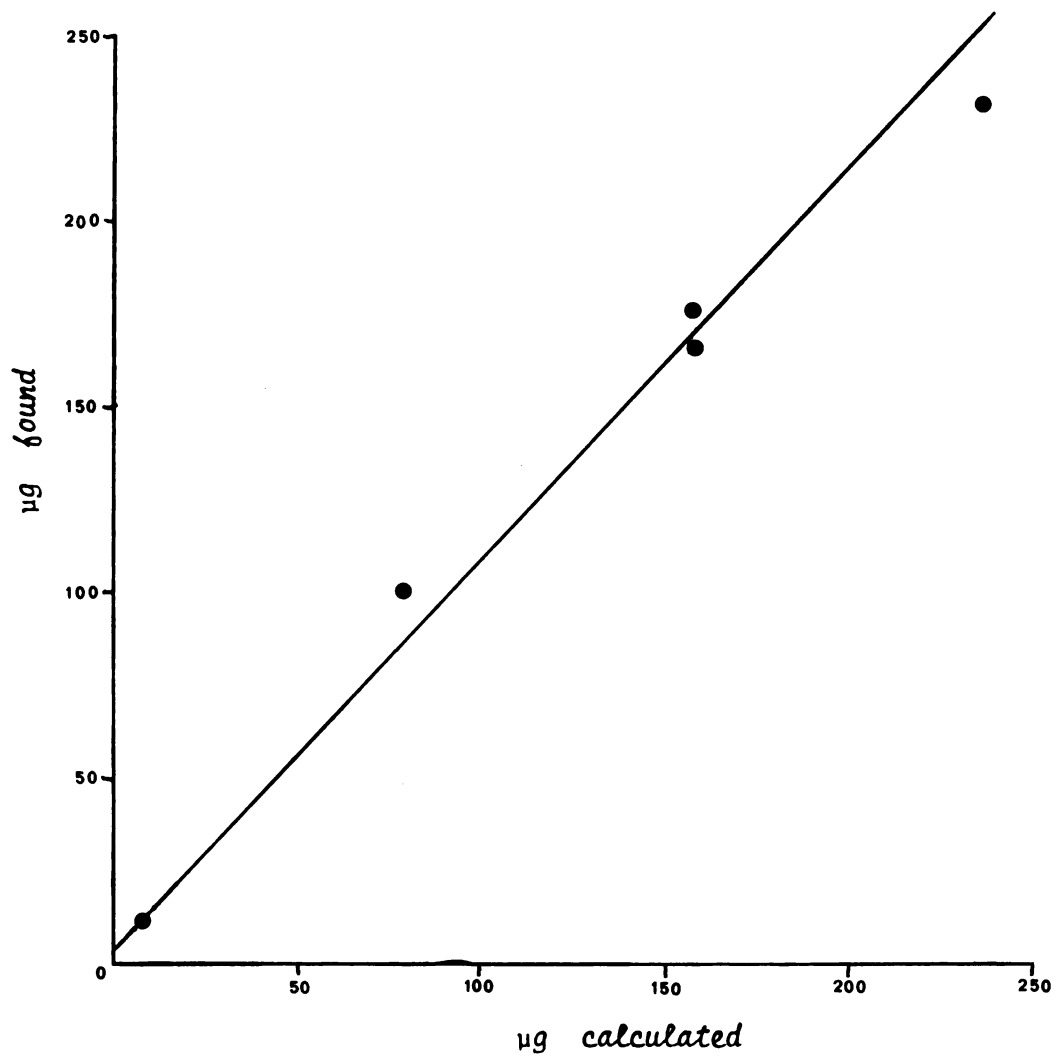


Fig. 34 5-Hydroxytryptophol standard curve determined by CIMS assay.

Indoleacetic Acid

A standard curve for quantifying indoleacetic acid was obtained in a manner similar to that for tryptophol and 5-hydroxytryptophol. Known amounts of IAA and d₇-IAA were added to buffer solutions (pH 7.4). The solutions were first extracted at pH 7.4 and then at pH 1.8. The acid pH extracts were concentrated to dryness, redissolved in acetone and treated with diazomethane to produce the methyl esters. Analysis of the C.I. mass spectra allowed for ratio determination (MH⁺ 190/197). Figure 35 shows the results of plotting μg IAA found experimentally vs μg IAA calculated to have been added. The relationship is linear over a range of 10-250 μg . This would correspond to a range of 0.15 - 3.75 mg/24 hr. urine.

Solutions of IAA and d₇-IAA consisted of known amounts weighed to 0.01 mg \pm 0.005 mg on a Cahn Electrobalance and were dissolved in 0.2 M phosphate buffer, pH 7.4 and were of the following concentrations:

indoleacetic acid	0.70 $\mu\text{g}/\text{ml}$
d ₇ -indoleacetic acid	0.99 $\mu\text{g}/\text{ml}$

1. The first part of the document discusses the importance of maintaining accurate records of all transactions.

2. It then outlines the various methods used to collect and analyze data, including surveys and interviews.

3. The next section describes the results of the study, highlighting the key findings and their implications.

4. Finally, the document concludes with a summary of the research and suggestions for future work.

5. The following table provides a detailed breakdown of the data collected during the study.

6. This section includes a discussion of the limitations of the study and the potential for bias.

7. The authors also discuss the ethical considerations that guided the research process.

8. In addition, the document provides a list of references for further reading on the topic.

9. The following section contains a list of appendices that provide additional information related to the study.

10. Finally, the document includes a list of contact information for the authors and their institutions.

11. The authors would like to thank the following individuals for their assistance and support during the study:

12. This research was supported by a grant from the National Science Foundation.

13. The authors have no conflicts of interest to disclose.

14. The data used in this study are available upon request.

15. The authors would like to thank the following individuals for their assistance and support during the study:

16. This research was supported by a grant from the National Science Foundation.

17. The authors have no conflicts of interest to disclose.

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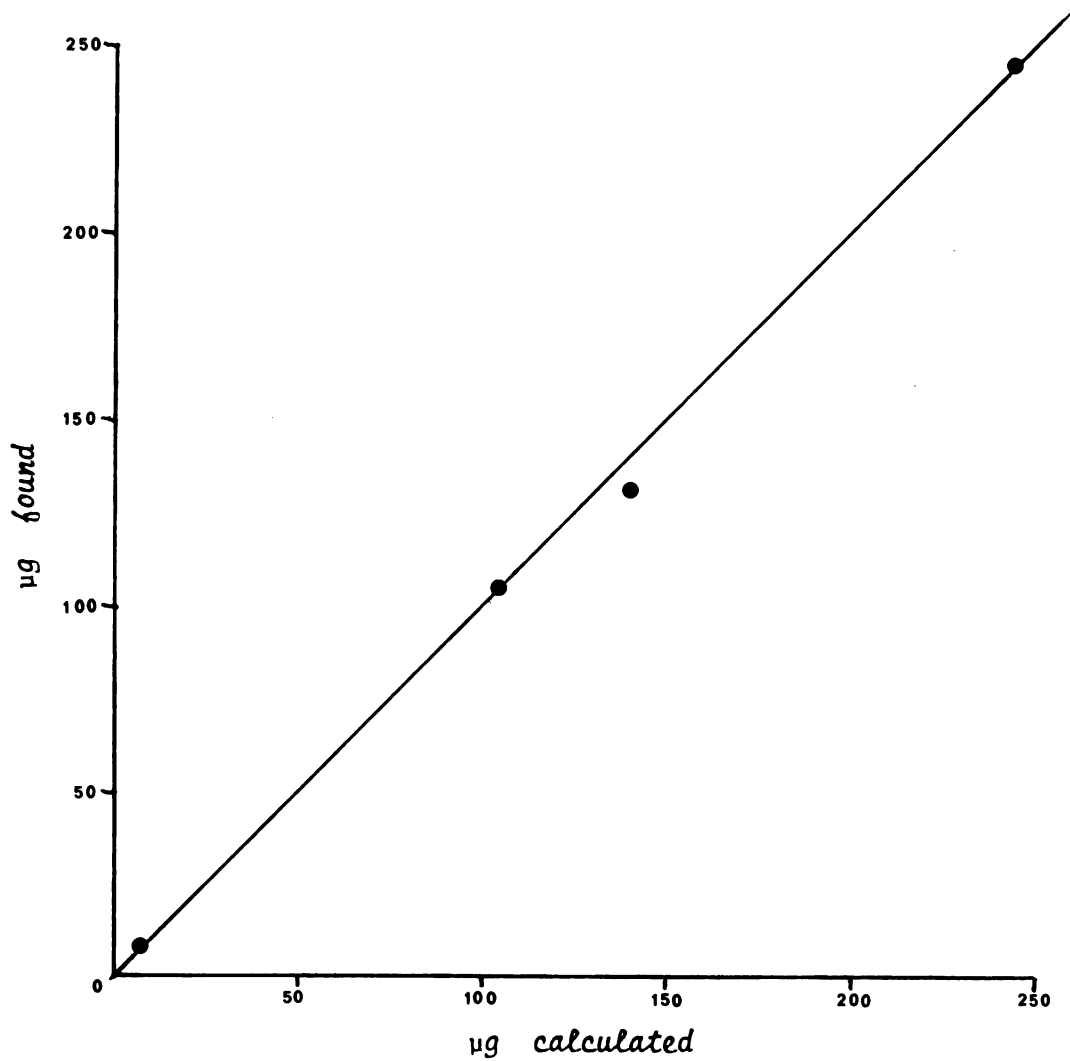


Fig. 35

IAA standard curve determined by CIMS assay of IAA methyl ester.

Part IV

Biological

A. In Vivo Experiments Employing Stable - Isotope Labeled Tryptophan

In Vivo Studies Using Stable Isotopically Labeled Tryptophan

Introduction

In order to study more fully the metabolism of tryptophan by patients with carcinoid tumor and to realize more fully the potential of C.I.M.S. as an analytical tool, the use of a "label" that could be detected in the mass spectrum was evaluated. Stable isotopically labeled tryptophan would be administered to a patient in the form of an oral load. However, to do this, certain parameters of this dosage had to be determined. The University of California Committee on Human Experimentation has strict guidelines concerning the administration of any compound to any person, patient or volunteer control subject.

It was thus necessary to conduct animal studies to determine absolute recovery of administered compound. In addition, the potential dangers of administering to patients a 50:50 mixture of deuterium labeled L-tryptophan and "cold" L-tryptophan in order to achieve doublets for metabolites in the mass spectra had to be determined. Although the administration of this ratio of labeled to "cold" compound may be suitable for exogenous compounds such as drugs, the body pool of an endogenous compound such as an essential amino acid is so large that the label may become too dilute to detect. I had to know whether I had to administer a larger dose of labeled tryptophan.

1. The first part of the text discusses the importance of maintaining accurate records of all transactions and activities related to the business.

2. It then goes on to describe the various methods used to collect and analyze data, including surveys, interviews, and focus groups.

3. The next section covers the process of identifying and defining the research objectives and the scope of the study.

4. This is followed by a discussion of the different types of data that can be collected, such as primary and secondary data.

5. The text then describes the various methods used to collect data, including surveys, interviews, and focus groups.

6. It also discusses the importance of ensuring the reliability and validity of the data collected.

7. The next section covers the process of analyzing the data and drawing conclusions from the results.

8. This is followed by a discussion of the different types of reports that can be generated from the data.

9. The text then describes the various methods used to present the data, including tables, charts, and graphs.

10. Finally, it discusses the importance of interpreting the results and making recommendations based on the findings.

11. The text concludes by emphasizing the importance of maintaining accurate records and using the data to make informed decisions.

12. It also discusses the importance of ensuring the reliability and validity of the data collected.

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20. Finally, it discusses the importance of interpreting the results and making recommendations based on the findings.

I could not answer the questions concerning the effects of a stable isotopically labeled amino acid on the body other than a general discussion of kinetic isotope effects on metabolic processes (38,133,144) but I could document the effects of a loading dose of tryptophan.

In control subjects, a load of 100 mg/kg may produce some headache, CNS depression, confusion, disorientation, and nausea. These effects are mild and short (1-2 hr) and no harmful effects have been reported in the literature (115,116).

Patients with carcinoid tumor usually exhibit the same symptoms after ingestion of a tryptophan load. However, some exhibit a transient exacerbation of the carcinoid syndrome - increased flush, hypotension and diarrhea. However, for some patients the difference in quantities of metabolites produced may relate to an absolute increase in urinary metabolites after tryptophan loading. Before any load could be administered, the benefits to the patient had to be weighed against any possible discomfort that might be experienced.

The first part of the document discusses the importance of maintaining accurate records of all transactions and activities. It emphasizes that proper record-keeping is essential for ensuring transparency and accountability in financial operations. This section also outlines the various methods and tools used to collect and analyze data, highlighting the need for consistency and precision in data entry and reporting.

The second part of the document focuses on the implementation of internal controls and risk management strategies. It details the process of identifying potential risks and developing effective mitigation plans to minimize their impact on the organization. This section also discusses the role of internal audits in monitoring and evaluating the effectiveness of these controls, ensuring that the organization remains compliant with relevant regulations and standards.

The third part of the document addresses the importance of communication and collaboration in achieving organizational goals. It emphasizes the need for clear and concise communication channels, as well as the importance of fostering a culture of transparency and open dialogue. This section also discusses the role of leadership in setting the tone for the organization and ensuring that all employees are aligned with the organization's mission and vision.

The final part of the document provides a summary of the key findings and recommendations. It highlights the areas where the organization has made significant progress and identifies the key challenges that remain. The document concludes with a call to action, urging the organization to continue to strive for excellence and to embrace change as a catalyst for growth and innovation.

In Vivo Studies: Rat

A search of the literature showed that varying amounts of a loading dose of tryptophan could be accounted for in the urine, feces and as CO_2 . A review by Leklem on ^{14}C -tryptophan metabolism in several animal species with and without a load shows that for the rat, between 2.4 and 15.9% of an administered dose of L or DL tryptophan can be accounted for in the urine (116). An additional 5-36% can be accounted for as $^{14}\text{CO}_2$.

In man, $^{14}\text{CO}_2$ output ranged from 3.8 to 14.7% of the dose while 25-28% of the dose appeared in the urine in 24 hours. Differences in output of $^{14}\text{CO}_2$ are due in part to different positions of the label. In addition, loading studies done in humans show that 0.003 to 0.2% of the dose was accounted for as urinary 5-HT whereas urinary 5-HIAA accounted for 0.02 to .07% of the dose. Thus, the total amount of label that could be accounted for in 24 hr in humans ranged from a low of 28% to a high of 42% (115,116).

In my study using ^{14}C labelled L-tryptophan (3-position of side chain) administered with a load of L-TP intraperitoneally to a rat, 9.6% of the dose could be accounted for in the 24 hr urine. An additional 1.2% was found in the 48 and 72 hr urines and 0.46% in the 7 day feces. These values agree well with the literature value of 2.4 - 15.9% excreted in the urine. It was therefore clear from this and other reported experiments that I could account for no more than one-fourth the dose of a load of tryptophan as compounds excreted in the urine. An additional 10% of the dose would probably be excreted

1. The first part of the document discusses the importance of maintaining accurate records of all transactions.

2. It then outlines the various methods used to collect and analyze data from different sources.

3. The next section describes the challenges faced in data collection and how they can be overcome.

4. This is followed by a discussion on the ethical considerations surrounding data collection and analysis.

5. The final part of the document provides a summary of the key findings and conclusions.

6. The document concludes with a list of references and a bibliography.

7. The following table provides a detailed breakdown of the data collected during the study.

8. The data shows a clear trend of increasing activity over the period of the study.

9. This increase is attributed to a combination of factors, including improved data collection methods.

10. The results of the study have significant implications for the field of data analysis.

11. Further research is needed to explore the underlying causes of these trends.

12. The study also highlights the need for continued investment in data collection technology.

13. Overall, the findings of this study provide a valuable insight into the current state of data collection.

14. The data suggests that there is still much to be learned about the complexities of data collection.

15. The study also identifies several areas for future research and development.

16. The results of this study are consistent with previous research in the field.

17. The study provides a comprehensive overview of the current state of data collection.

as CO_2 but methods were not available to make this determination. it is apparent that a large percentage of the dose (and hence the deuterium) will remain in the body, perhaps incorporated into protein or perhaps, as niacin, into the co-enzymes NAD^+ and NADP^+ . The effect of a deuterium on tryptophan incorporated into protein or into the nicotinamide moiety of NAD^+ is not known. But this question of isotope effect coupled with the fact that a large amount (50%) of an administered dose will remain in the body for a significant time raises the question of the safety to the patient of administering large amounts of even a stable isotopically labeled amino acid. The question of the relative safety of administering a very small amount of radio label vs a large amount of stable label has not been answered. Too little is known of the long-term effect of heavy isotopes in living systems. Some studies (114,115) suggest harmful effects occur only at very high levels of isotope incorporation. But no studies have been done with stable isotopically labeled amino acids in humans. Until such studies are done, the safety of a load of this nature will be in question.

In Vivo Studies: Rabbit and Monkey

Studies of the efficacy of administration of a 50-50 mixture of labeled tryptophan and "cold" tryptophan were conducted in the rabbit and the monkey. In addition to noting if mass spectral doublets could be produced from this mixture, the obvious appearance of the animal was noted at various times in an attempt to demonstrate the onset and duration of easily observable side effects such as CNS stimulation or depression.

For both animals, food was withheld from 24 hr prior to the administration of the label until 6 hr after administration. Water was available at all times and the urine was collected in a pan which drained into a flask immersed in a dry ice bath. Urine was kept frozen until analysis. Analysis consisted of ethyl acetate extraction of acids and bases followed by derivatization and C.I.M.S. analysis.

In the rabbit study, a dose of 50mg/kg of 50%-²H-DL-TP was used. One half hour after intraperitoneal injection, the normally alert and active rabbit was sedate and lying on its side. At one hour this apparent sedation was minimal and after 4 hr the rabbit appeared normal. The monkey was administered a 100mg/kg dose via a stomach catheter. The monkey showed no observable CNS sedation or drowsiness and was just as alert and feisty as before administration.

Experimental

A male New Zealand white rabbit (3.08 kg) was placed in a wire-bottom cage with a pan beneath it. The pan drained into a flask immersed in a dry-ice/isopropanol bath. Urine was stored frozen until analyzed. Food was removed 24 hr prior to administration of the compounds. A dose (50 mg/kg) of 50% DL-tryptophan (Aldrich Chemical Co.) and 5-²H-DL-tryptophan in 20 ml 0.05 M sodium phosphate buffer, pH 7.8 was injected intraperitoneally. Food was returned 6 hr after injection. Water was always available. The 48 hr. urine was collected, an aliquot was taken and filtered and the pH adjusted to 12.0 and extracted with ethyl acetate to remove the basic and neutral compounds. The pH was then adjusted to 1.8 and extracted to remove the acidic compounds. The acidic compound containing solution was analyzed by C.I.M.S. by direct application to the mass spectrometer probe. The acids were also derivatized to the methyl esters with diazomethane and analyzed by C.I.M.S.

A Rhesus monkey (5.5 kg) was placed in a chair restraint which allowed for the collection of urine in a pan which drained into a flask immersed in a dry-ice/isopropanol bath. The urine was stored frozen until analyzed. Food was removed 24 hr prior to administration of the compounds. A dose (100 mg/kg) of 50% DL-tryptophan (Aldrich Chemical Co.) and 5-²H-DL-tryptophan in 100 ml 0.2 M sodium phosphate buffer, pH 7.4, was administered via a stomach catheter at a rate of 3 ml/min. Food was returned 6 hr after administration. Water was always available. Separate 24 hr (460 ml) and 48 hr urines were collected, and ethyl acetate extract containing the basic/neutral

The first part of the document discusses the importance of maintaining accurate records of all transactions. It emphasizes that every sale, purchase, and expense must be properly documented to ensure the integrity of the financial statements. This includes obtaining receipts for all purchases and providing detailed invoices for all sales. The document also highlights the need for regular reconciliation of bank accounts and the use of double-entry bookkeeping to maintain the balance sheet.

The second part of the document focuses on the classification of assets and liabilities. It provides a detailed breakdown of how different types of assets, such as cash, accounts receivable, inventory, and property, should be recorded and valued. Similarly, it explains how various liabilities, including accounts payable, loans, and accrued expenses, should be categorized and reported. The document also discusses the importance of depreciation for fixed assets and the amortization of intangible assets.

The third part of the document addresses the preparation of the income statement and the statement of equity. It outlines the steps for calculating net income, including the treatment of depreciation, amortization, and other non-cash items. It also explains how the income statement is used to determine the change in equity and how this information is reflected in the statement of equity. The document provides examples of how to calculate these figures and how to present them in a clear and concise manner.

The final part of the document discusses the importance of internal controls and the role of the auditor. It emphasizes that a strong internal control system is essential for preventing errors and fraud, and for ensuring the accuracy of the financial statements. It also explains the role of the auditor in providing an independent opinion on the financial statements and the importance of transparency and disclosure in financial reporting.

compounds and the acidic compounds were obtained. C.I.M.S. analyses of both the free acids and the methyl esters were conducted.

Another aliquot of monkey urine was studied by C.I.M.S. analysis according to the procedure outlined in the section describing the C.I.M.S. assay. Internal standards of d_4 -tryptophol (50.86 μg) and d_4 -5-hydroxytryptophol (70.85 μg) were added to 100 ml of urine. (These values compute to 228 $\mu\text{g}/24$ hr urine and 318 $\mu\text{g}/24$ hr respectively.) The pH was adjusted to 9.0 and extracted with three 50 ml portions of ethyl acetate to remove basic, neutral and phenolic compounds. After readjusting the pH to 2.0, the acidic compounds were removed by ethyl acetate extraction. The basic fraction was chromatographed on silica gel (Eastman) in solvent system B: $\text{CHCl}_3/\text{HOAc}/\text{MeOH}/\text{H}_2\text{O}$ (65:20:10:5), 1 hr, 9.0 cm, and the spots at the R_f corresponding to tryptophol and 5-hydroxytryptophol (0.85 and 0.50 respectively) were eluted with methanol and analyzed by C.I.M.S. A 0.1 ml aliquot of the acidic fraction was chromatographed first in solvent system A: acetone/isopropanol/water/ammonia (50:40:7:3), 2 hrs, 15.0 cm, and then in system B. The spots of R_f values corresponding to various indolic acids were eluted with methanol, derivatized to the methyl esters, deuterium standards added and analyzed by C.I.M.S.

1. The first step in the process of identifying a problem is to recognize that a problem exists. This is often done by comparing current performance with a desired state or goal. For example, a manager might notice that sales are declining or that customer satisfaction is low. Once a problem is identified, the next step is to define it more precisely. This involves determining the scope of the problem, its causes, and its effects. For instance, a manager might define a problem as "a 10% decrease in sales over the last quarter, primarily due to a loss of market share in the competitive market." This definition helps to narrow down the focus of the problem and provides a clear starting point for further investigation.

2. The second step in the process is to gather information about the problem. This involves collecting data and facts that are relevant to the problem. For example, a manager might gather data on sales trends, market conditions, and customer feedback. This information is then used to identify the underlying causes of the problem. For instance, a manager might discover that the loss of market share is due to a combination of factors, including increased competition, changes in consumer preferences, and a lack of innovation in the product line. This information is crucial for developing an effective solution.

3. The third step in the process is to analyze the information gathered. This involves identifying the key factors that are contributing to the problem and determining their relative importance. For example, a manager might analyze the data to identify the most significant causes of the sales decline. This analysis might reveal that the most significant cause is a lack of innovation in the product line. This information is then used to develop a list of potential solutions. For instance, a manager might develop a list of solutions that includes increasing R&D investment, improving customer service, and launching new products.

4. The fourth step in the process is to evaluate the potential solutions. This involves comparing the different solutions and determining which one is the most effective and feasible. For example, a manager might evaluate the solutions based on their expected impact on sales, their cost, and their feasibility. This evaluation might reveal that increasing R&D investment is the most effective and feasible solution. This information is then used to select a solution to implement.

5. The fifth step in the process is to implement the selected solution. This involves putting the solution into action and monitoring its progress. For example, a manager might implement the solution by increasing R&D investment and launching new products. The manager would then monitor the progress of the solution and make adjustments as needed. This step is crucial for ensuring that the solution is effective and that the problem is resolved.

6. The sixth step in the process is to evaluate the results of the solution. This involves comparing the current performance with the desired state and determining whether the problem has been resolved. For example, a manager might evaluate the results of the solution by comparing sales trends and customer satisfaction with the desired state. This evaluation might reveal that the problem has been resolved and that sales are increasing and customer satisfaction is high. This information is then used to identify any remaining issues and to develop a plan for the future.

Results and Discussion

In both experiments, C.I.M.S. analysis revealed a complex mixture of compounds with no readily discernable doublets. It is obvious that a ratio greater than 50:50 (labeled to unlabeled) must be administered for doublets to appear in the mass spectrum.

The use of stable - isotopically labeled tryptophol and 5-hydroxytryptophol as internal standards was successful in determining the quantities of these two compounds excreted by the monkey in 24 hr. The amount of tryptophol measured was 292 $\mu\text{g}/24$ hr and the amount of 5-hydroxytryptophol was 193 $\mu\text{g}/24$ hr.

Part IV

B. Assay of Patient Urinary Metabolites of Tryptophan

Identification and Quantification of Urinary Indole Metabolites
Excreted by Patients with Carcinoid Tumor

Introduction

The main focus of my work was detection and quantification of various urinary indolic tryptophan metabolites excreted by patients with carcinoid tumor. Previous assays were concerned only with 5-HIAA or IAA urinary levels both with and without an oral tryptophan load (14,21,117). Williams and associates (14) were able to demonstrate differences in amounts of 5-HIAA and IAA excreted by patients with bronchial carcinoid, ileal carcinoid and what they termed type IV carcinoid. Table 12 shows those results and includes values found in normals both with and without tryptophan loading.

Beside 5-HIAA and IAA, the urine of carcinoid patients were examined to determine the presence and/or amount of the following indolic tryptophan metabolites: indolelactic acid (ILA), indolepyruvic acid (IPyA), indolepropionic acid (IPA), indolebutyric acid (IBA), indoleacrylic acid (IAcryA), 5-hydroxyindoleacrylic acid (5-HIAcryA) tryptophol (TOH), 5-hydroxytryptophol (5-HTOH), N-acetyltryptophan, N-acetyl-5-hydroxytryptophan, 5-hydroxytryptophan (5-HTP) and tryptophan (TP).

The principal methods of detection and quantification were thin layer chromatography, gas-liquid partition chromatography (glpc) and chemical ionization mass spectrometry.

Table 12

Total amounts of Indoleacetic acid (IAA) and 5-Hydroxyindoleacetic acid (5-HIAA) excreted in the urine in 24 hr by normal and carcinoid patients before and after an oral load of L-tryptophan (from Williams, et al, 14)

	Bronchial	Ileal	Type IV	Normal
Total IAA Baseline mg/24 hr	0.6	17.63	10.8	4.71 (14) 3.1-8.1 (21) 8.0-17.4 (117)
Total IAA Loading mg/24 hr	22.2	60.77	22.61	15.94
Total 5-HIAA Baseline mg/24 hr	755	381	3.36	3.07
Total 5-HIAA Loading mg/24 hr	1179.6	348.3	4.29	1.89

Total IAA Baseline, 24 hr. carcinoid 7-16.4 (21)

1. The first part of the document discusses the importance of maintaining accurate records of all transactions and activities. It emphasizes that this is essential for ensuring transparency and accountability in the organization's operations.

2. The second part of the document outlines the various methods and tools used to collect and analyze data. It highlights the need for consistent and reliable data collection processes to support effective decision-making.

3. The third part of the document focuses on the role of technology in data management and analysis. It discusses how modern software solutions can streamline data collection, storage, and reporting, thereby improving efficiency and accuracy.

4. The fourth part of the document addresses the challenges associated with data management, such as data quality, security, and privacy. It provides strategies to mitigate these risks and ensure that data is used responsibly and ethically.

5. The fifth part of the document discusses the importance of data governance and the establishment of clear policies and procedures. It stresses that a strong data governance framework is crucial for maintaining data integrity and compliance with regulatory requirements.

6. The sixth part of the document explores the benefits of data-driven decision-making and how it can lead to improved organizational performance. It provides examples of how data analysis can identify trends, opportunities, and areas for improvement.

7. The seventh part of the document concludes by summarizing the key points discussed and reiterating the importance of a data-centric approach. It encourages the organization to continue investing in data management and analysis to stay competitive in the market.

Materials and Methods

Patient Samples

Patient urines were obtained and extracted according to the procedure described earlier in the section dealing with the CIMS assay technique.

Thin Layer Chromatography

Two dimensional thin layer chromatography was conducted following the procedure of Haworth and Walmsley (118) using the following solvent systems: Solvent A, acetone/isopropanol/water/ammonia (50:40:7:3); solvent B, chloroform/acetic acid/methanol/water (65:20:10:5). Solvents A and B were allowed to equilibrate in glass chambers for 30 min prior to development. Samples were spotted on Eastman Silica Gel Chromagram sheets 6060 (with fluorescent indicator), at the origin, 1.5 cm from each side. The plate was developed 2 hr (15.0 cm) in solvent A, dried at room temperature for 1 hr then developed in solvent B 1 hr (9.0 cm) and dried at room temperature. Rf values were determined by uv identification and aided by treatment with 1% Ehrlich's solution.

Gas-Liquid Partition Chromatography

GLPC was conducted on a Varian 2100 chromatograph using a 3 ft glass U-shaped column packed with 2% OV-1 on Chromosorb GHP (80-100 mesh). Compounds and extracts were either silylated with BSTFA (Pierce Chemical Co.) or treated with etherial diazomethane.

Chemical Ionization Mass Spectrometry

CIMS was performed on an AEI MS-9 instrument fitted for chemical ionization. Compounds in solution and extracts were applied to a direct insertion probe. Reactant gases included methane, isobutane and isopropyl ether. Ionization temperatures ranged from 150° to 300° C. Compounds or extracts were analyzed without derivatization and/or after treatment with BSTFA or diazomethane.

1. *Pharmaceutical Innovation and the Role of Patents*

2. *The Impact of Patent Law on Drug Development*

3. *Patent Law and the Challenge of Generic Drugs*

4. *The Role of Patents in the Pharmaceutical Industry*

5. *Patent Law and the Challenge of Biotechnology*

6. *The Impact of Patent Law on the Pharmaceutical Industry*

7. *Patent Law and the Challenge of仿制药*

8. *The Role of Patents in the Pharmaceutical Industry*

9. *Patent Law and the Challenge of仿制药*

10. *The Impact of Patent Law on the Pharmaceutical Industry*

Results and Discussion

Table 13 shows TLC Rf values for several indolic tryptophan metabolites. Table 14 shows GLPC retention times for selected metabolites. Table 15 is a list of urinary acids and bases, including tryptophan metabolites, arranged according to increasing molecular weight. Table 16 is a list of indolic acids that were considered as possible urinary metabolites, arranged according to the molecular weight of their methyl esters or methyl ethers.

Figures 36,37 and 38 show C.I.M.S. of extracts of patients' 24 hr urines. The extracts were applied directly to the mass spectrometer probe without treatment to produce derivatization. In fig. 36 (patient D.B.-I urinary acid extract), spectra a through f were recorded at correspondingly higher temperatures. The peak for IAA (MH^+ 176) can be seen to increase from spectra a and then decrease. The peak for 5-HIAA (MH^+ 192) is relatively small. This corresponds well with the 5-HIAA assay results obtained by the clinical laboratory which showed a normal level of 5-HIAA excreted in a 24 hr urine.

This patient was being examined for possible carcinoid tumor and did not have any biopsy proven tumor. Patients M.A. and P.L. had proven carcinoid tumors. Patient M.A. had laboratory assayed values as high as 800 mg 5-HIAA per 24 hr. urine. As seen in fig. 37, the peak for 5-HIAA is relatively large. Patient P.L. (fig. 38) also shows a significant peak for 5-HIAA in the C.I.M.S. of a urinary extract and had laboratory assayed values as high

as 300 mg 5-HIAA per 24 hr urine. These three spectra are examples of how C.I.M.S. was used to initially and rapidly screen the urine of patients for acidic and neutral indolic metabolites.

Both GLPC and CIMS were used to analyze the urine of several patients and served to establish the identity of a previously undetected metabolite of tryptophan. An extract of urinary acids from patient C.O. was analyzed by isobutane C.I.M.S. Table 17 lists the most significant peaks in the spectra obtained when the temperature of the mass spectrometer ionization chamber was slowly increased from 150° to 310°. The peaks for IAA (MH^+ 176) and 5-HIAA appeared as well as a peak at MH^+ 204. Subsequent GLPC analysis of the urine extract (treated with BSTFA to form the silylated products), enabled identification of one of the peaks as indolepyruvic acid (mw 203) by comparison of retention time with authentic indolepyruvic acid (fig. 39, table 14). Collection of the peak in question from the gas chromatograph, hydrolysis of the silyl groups in aqueous methanol followed by CIMS showed the GLPC peak to be of mw 203. Thus indolepyruvic acid was shown to exist in the urine of patient C.O., who has diagnosed carcinoid tumor.

Subsequent to the initial demonstration of the utility of CIMS in screening carcinoid urine for metabolites, the mass spectrometer was used to focus on and continuously monitor two peaks representing different masses. The exact method has been previously described in the section on assay technique. Table 18 shows the results of monitoring the masses of IAA and 5-HIAA of urinary extracts of several patients. Table 19 shows the baseline levels of IAA and

1. *What is the main purpose of the document?*

The main purpose of the document is to provide a comprehensive overview of the project's progress and to identify the key challenges and opportunities that we are currently facing.

2. *What are the key findings of the research?*

The key findings of the research are that the current market conditions are highly volatile and that there is a significant risk of a global economic downturn. This has led to a decrease in consumer spending and a rise in unemployment rates.

3. *What are the main challenges facing the organization?*

The main challenges facing the organization are the high level of competition and the limited resources available to us.

4. *What are the main opportunities for the organization?*

The main opportunities for the organization are the growing demand for our products and services and the potential for new markets.

5. *What are the main risks to the organization?*

The main risks to the organization are the high level of competition and the limited resources available to us.

6. *What are the main recommendations for the organization?*

The main recommendations for the organization are to focus on our core products and services and to expand our marketing efforts.

7. *What are the main conclusions of the document?*

The main conclusions of the document are that the current market conditions are highly volatile and that there is a significant risk of a global economic downturn.

8. *What are the main recommendations for the future?*

The main recommendations for the future are to focus on our core products and services and to expand our marketing efforts.

9. *What are the main conclusions of the document?*

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10. *What are the main recommendations for the future?*

The main recommendations for the future are to focus on our core products and services and to expand our marketing efforts.

5-HIAA according to type of carcinoid tumor and ratios of IAA to 5-HIAA (14). Several IAA to 5-HIAA ratios of patients determined by CIMS constant ion monitoring are shown in figures 40 to 51. The ratios obtained for patients D.B.-I and M.A. correspond very well with the information obtained from the initial CIMS screening (figs. 36 and 37). The initial screening showed the urine of patient D.B.-I to contain more IAA than 5-HIAA. This was confirmed by the constant ion monitoring technique (fig. 40). Initial screening of patient M.A.'s urine showed it to contain an excessive amount of 5-HIAA which was confirmed by constant ion monitoring (fig. 48).

Unfortunately, as described previously, the constant ion monitoring technique was not refined enough to give reproducible results. The error in ratio determination was great enough for me to eliminate this method for patient metabolite assay.

1. **Introduction**

The purpose of this report is to analyze the impact of the COVID-19 pandemic on the global economy and to provide recommendations for recovery. The report is structured as follows:

- 2. **Methodology**
- 3. **Results**
- 4. **Discussion**
- 5. **Conclusion**

The data for this report was collected from various sources, including government reports, academic journals, and news articles. The analysis was conducted using a combination of quantitative and qualitative methods.

The results of the analysis show that the COVID-19 pandemic has had a significant negative impact on the global economy. There has been a sharp decline in GDP, a rise in unemployment, and a decrease in consumer spending. However, there are signs of recovery in some sectors, such as technology and e-commerce.

The discussion section of the report explores the reasons for these trends and the potential for a long-term recovery. It also discusses the role of government intervention and the importance of international cooperation.

In conclusion, the COVID-19 pandemic has presented a major challenge to the global economy. While there are signs of recovery, it is clear that a long and difficult road lies ahead. The report provides a comprehensive overview of the current situation and offers recommendations for how to best navigate this crisis.

Table 13

Thin layer chromatography of standard indoles on silic gel developed
 15.0 cm. in solvent A (acetone/isopropanol/water/ammonia 50:40:7:3)
 and 9.0 cm. in solvent B (chloroform/acetic acid/methanol/water 65:20:10:5)

<u>Compound</u>	<u>R_f</u>		<u>color w/ 1% Ehrlichs</u>
	<u>A</u>	<u>B</u>	
Indoleacetic acid	.39	.90	yellow
5-Hydroxyindoleacetic acid	.39	.77	blue
Indolepropionic acid	.19	.47	none
Indolebutyric acid	.28	.47	green
Indolelactic acid	.37	.57	yellow
Indolepyruvic acid	.33	.67	yellow
Indoleacrylic acid	.46	.89	green
5-Methoxyindoleacrylic acid	.26	.86	pink- orange
Tryptophol	.77	.85	yellow
5-Hydroxytryptophol	-	.50	green-yellow
N-acetyltryptophan	.24	.75	purple
N-acetyl-5-hydroxytryptophan	.26	.75	blue

Table 14

Gas chromatographic retention times of various indolic acids chromatographed with a varian 2100 G.C. on a 3 ft column of 2% OV-1 on Chromosorb GHP (80-100 mesh). Column conditions were: oven, 180°; flash heater, 210°; detector, 230°; N₂ flow, 60 ml/min. All compounds were silylated with BSTFA (Pierce Chem. Co.).

<u>Compound</u>	<u>Retention time (min.)</u>
Indoleacetic acid	2.4
L-Tryptophan	7.5
5-Hydroxyindoleacetic acid	8.25
Indoleacrylic acid	13.5
Indolepyruvic acid	18.4
5-Hydroxytryptophan	22.5

1. The first step in the process of identifying a problem is to recognize that a problem exists. This is often done by comparing current performance with a desired state or goal. Once a problem is identified, the next step is to define the problem more precisely. This involves determining the scope of the problem, the resources available, and the constraints that may be present. The third step is to analyze the problem to determine its causes. This is often done by using tools such as fishbone diagrams or the 5 Whys technique. The final step is to develop and implement a solution. This involves identifying potential solutions, evaluating them, and then implementing the chosen solution. It is important to monitor the results of the solution and make adjustments as needed.

- Identify the problem: The first step is to identify the problem. This involves recognizing that a problem exists and defining it in terms of its symptoms and the impact it is having on the organization.
- Analyze the problem: The next step is to analyze the problem. This involves determining the causes of the problem and the resources available to solve it.
- Develop a solution: Once the problem has been analyzed, the next step is to develop a solution. This involves identifying potential solutions and evaluating them based on their feasibility and effectiveness.
- Implement the solution: The final step is to implement the solution. This involves putting the chosen solution into action and monitoring its progress.
- Evaluate the results: The final step is to evaluate the results of the solution. This involves comparing the current performance with the desired state and making adjustments as needed.

2. The process of identifying a problem is a continuous one. It is important to regularly monitor performance and to be open to the possibility of new problems. This is often done through the use of key performance indicators (KPIs) and regular reporting. It is also important to encourage employees to report problems and to create a culture of transparency and accountability.

Table 15

Common and suspected urinary acids and bases arranged according to molecular weight.

<u>Compound</u>	<u>mw</u>	<u>Compound</u>	<u>mw</u>
urea	60	tyrosine	181
creatinine	113	homovanilic acid	182
indole	117	4-pyridoxic acid	183
nicotinamide	122	epinephrine	183
nicotinic acid	123	indoleacrylic acid	187
5-hydroxyindole	133	indolepropionic acid	189
penylacetic acid	136	kynurenic acid	189
anthranilic acid	137	5-hydroxyindoleacetic acid	191
tyramine	137	dopa	197
p-hydroxyphenylacetic acid	152	indolepyruvic acid	203
3-hydroxyanthranilic acid	153	indolebutyric acid	203
dopamine	153	5-hydroxyindoleacrylic acid	203
tryptamine	160	tryptophan	204
tryptophol	161	6-hydroxykynurenic acid	205
phenylpyruvic acid	164	indolelactic acid	205
phenylalanine	165	5-methoxyindole acetic acid	205
phenyllactic acid	166	xanthurenic acid	205
uric acid	168	Kynurenine	208
homogentisic acid	168	N-acetyl-5-hydroxytryptamine	218
		5-hydroxyindolepyruvic acid	219

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indoleacetic acid	175	5-hydroxytryptophan	220
5-hydroxytryptamine	176	5-hydroxyindolelactic acid	221
5-hydroxytryptophol	177	3-hydroxykynurenine	224
hippuric acid	179	melatonin	232
p-hydroxyphenylpyruvic acid	180		

Table 16

Molecular weights of methyl esters and methyl ethers of various indolic acids

<u>Compound</u>	<u>mw</u>	<u>MH</u>
5-Hydroxyindole ME	147	148
IAA·ME	189	190
5-HTOH· ME	191	192
IAcryA· ME	201	202
IPA· ME	203	204
IPyA· ME	217	218
IBA· ME	217	218
5-HIAA·ME·ME	219	220
ILA· ME	219	220
5-HIAcryA· ME· ME	231	232
5-HIPyA· ME· ME	247	248
5-HILA· ME· ME	249	250

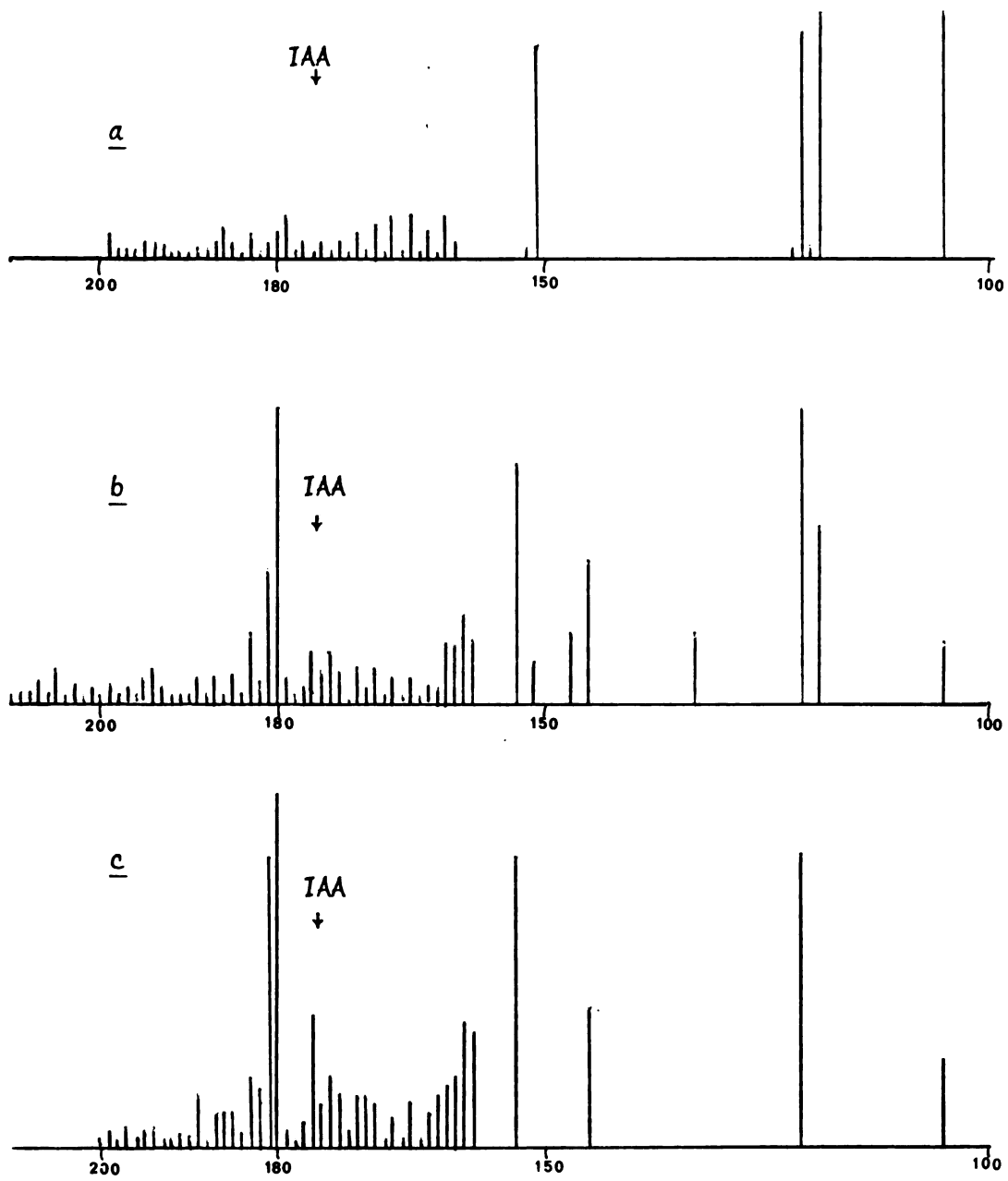


Fig. 36 Isobutane CIMS of patient D.B.-I urinary acids.
(continued on the next page)

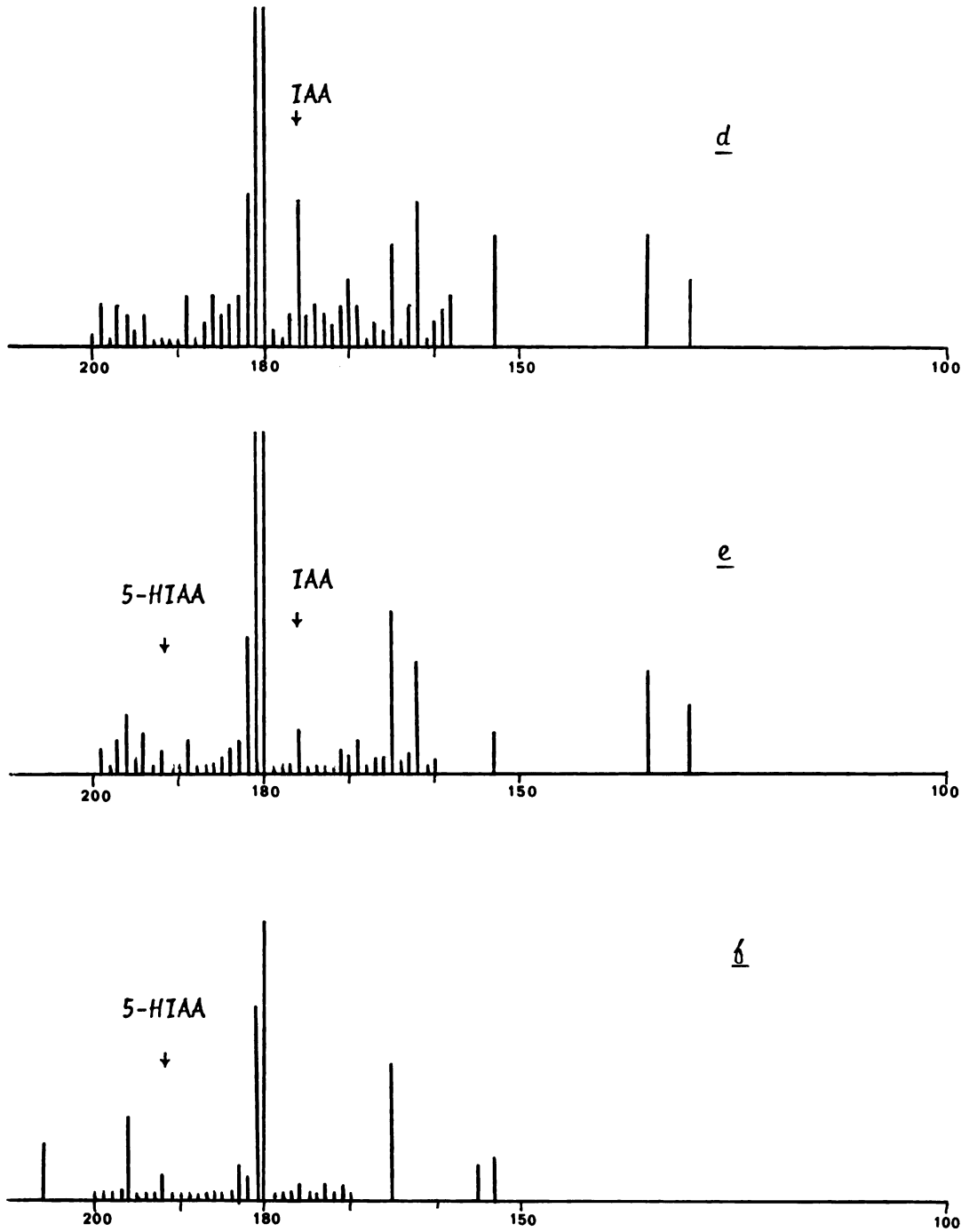


Fig. 36 Continuation of isobutane CIMS of patient D.B.-I urinary acids.

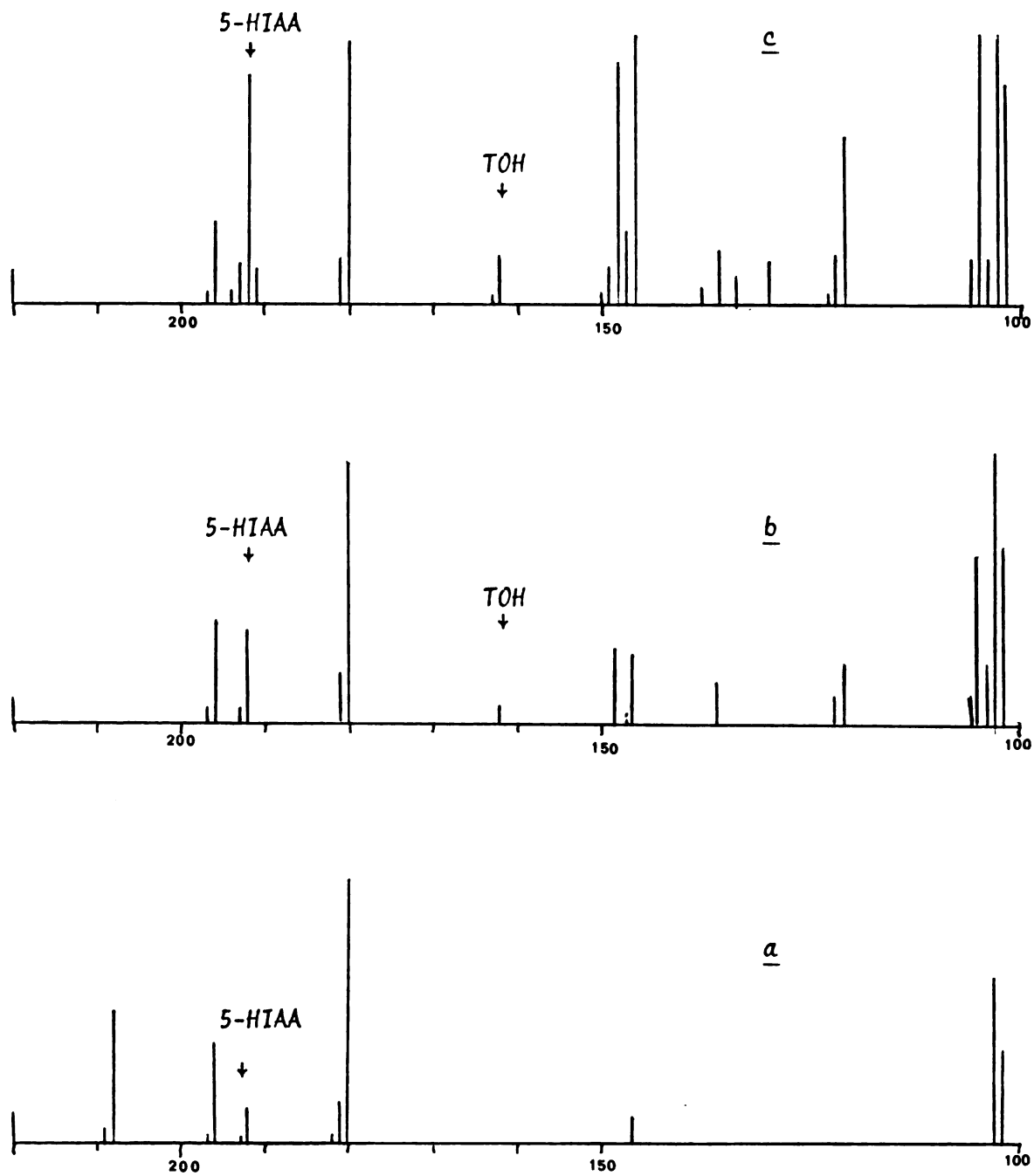


Fig. 37 Isopropyl ether CIMS of patient M.A. urinary acids and neutral compounds.

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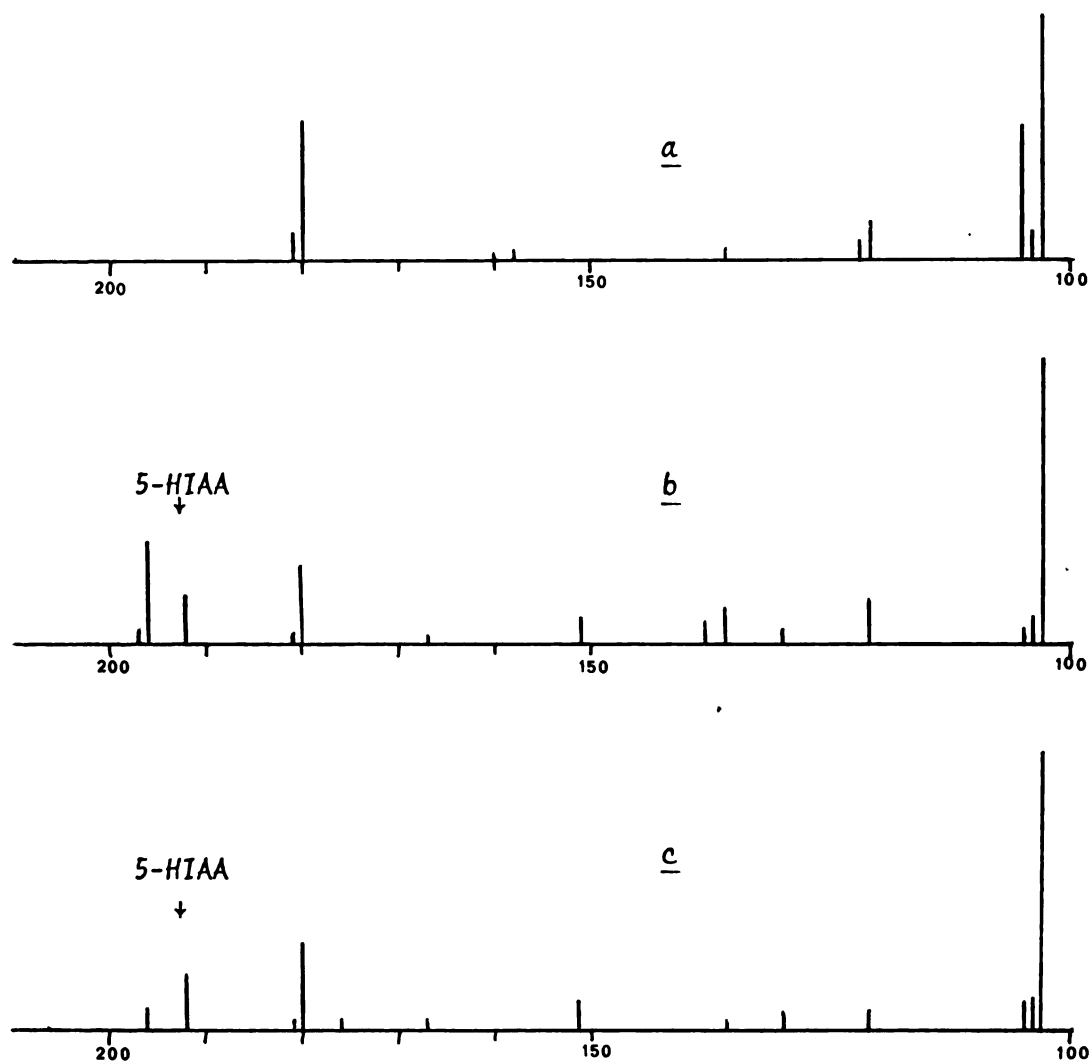


Fig. 38 *Isopropyl ether CIMS of patient P.L. urinary acids.*



Table 17

Chemical Ionization mass spectrum (isobutane) of patient C.O. urinary acidic and neutral compounds extracted into ethyl acetate at pH 2.0

<u>Spectrum #</u>	<u>Temp. Ion. chamber, °C</u>	<u>Mass</u>			
1.	150	391	10.	240	247
		148			192
		121			148
		174			
		175			
2.	160	391	11.	250	247
		148			192
		121			148
3.	170	390	12.	260	247
		179 IAA			204 IPyA
		121			192
					148
					146
4.	180	192 5-HIAA	13.	270	247
		176 IAA			204
5.	190	192	14.	280	247
		180			148
		176			
		148			148

1. The first step in the process of identifying a problem is to define the problem clearly.

2. The second step is to identify the causes of the problem.

3. The third step is to identify the effects of the problem.

4. The fourth step is to identify the stakeholders who are affected by the problem.

5. The fifth step is to identify the resources available to solve the problem.

6. The sixth step is to identify the constraints on the solution.

7. The seventh step is to identify the objectives of the solution.

8. The eighth step is to identify the alternatives available to solve the problem.

9. The ninth step is to identify the risks associated with each alternative.

10. The tenth step is to identify the best alternative.

11. The eleventh step is to implement the solution.

12. The twelfth step is to monitor the progress of the solution.

13. The thirteenth step is to evaluate the results of the solution.

14. The fourteenth step is to document the solution.

15. The fifteenth step is to communicate the solution to the stakeholders.

16. The sixteenth step is to review the solution.

17. The seventeenth step is to update the solution as needed.

18. The eighteenth step is to close the project.

19. The nineteenth step is to reflect on the project.

20. The twentieth step is to share the lessons learned.

6.	200	192		146
		180		
		176	15. 290	247
		148		201
				148
7.	210	247		146
		192		
		176	16. 300	201
		163		148
				140
8.	220	247		
		192	17. 310	146
				148
9.	230	247		
		192		
		148		

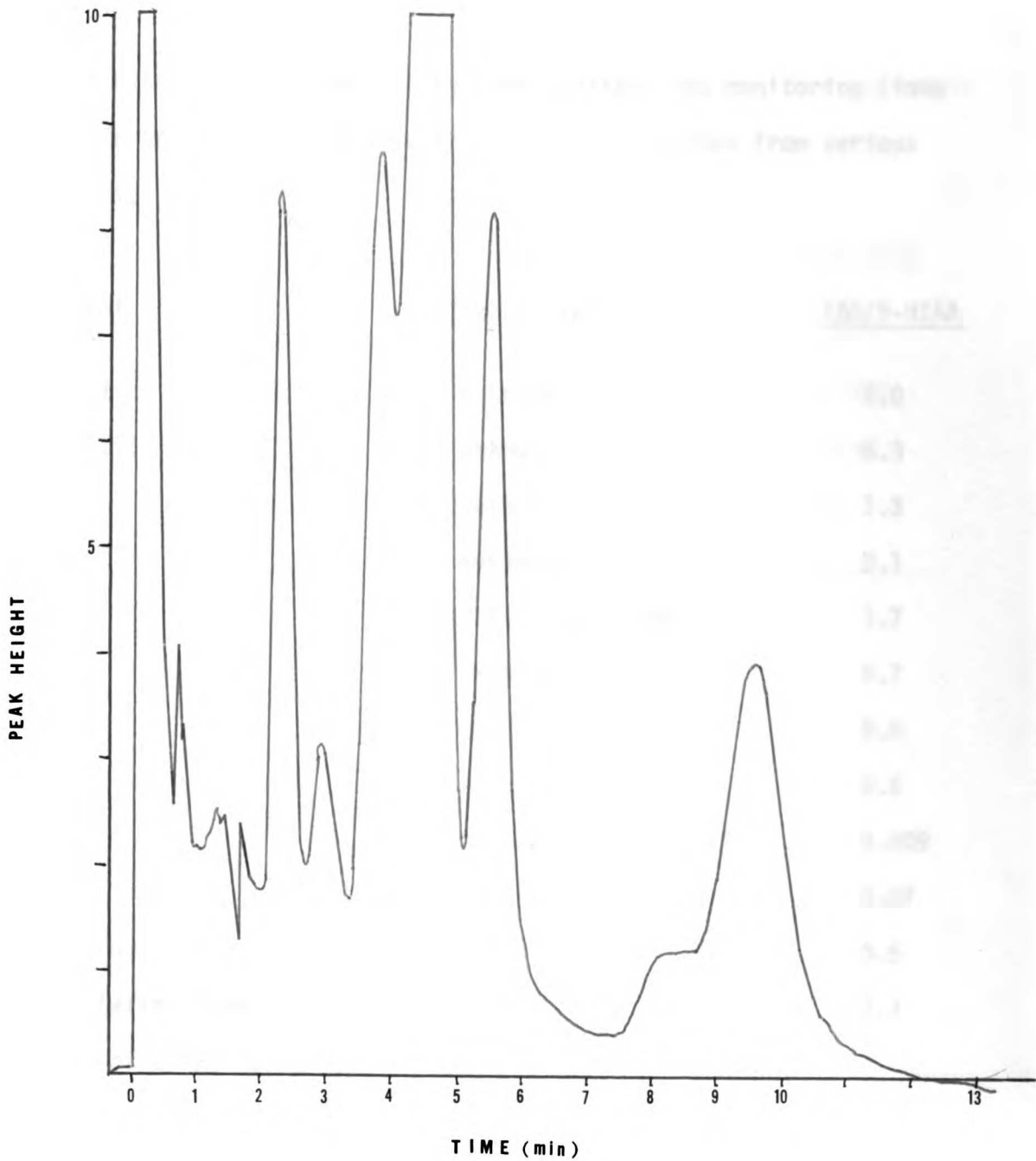


Fig. 39 Gas-Liquid Chromatograph of patient C.O. urinary acids treated with BSTFA.

Table 18

IAA/5-HIAA ratios determined by CIMS constant ion monitoring (ions m/e 176 and 192) of acidic extracts of 24 hr urines from various patients

<u>Patient</u>	<u>Disease state</u>	<u>IAA/5-HIAA</u>
D.B. I	unknown	2.0
D.B. III	unknown	6.3
C.L.	unknown	1.3
D.K.	mastocytosis	2.1
H.O.	pheochromocytoma	1.7
R.S.	unknown	0.7
P.E.	carcinoid	0.6
P.L.	carcinoid	0.6
M.A.	carcinoid (bronchial)	0.009
S.T.	unknown	0.07
R.M. (no load)	normal	0.6
R.M. (after loading dose of L-TP, 100mg/kg)	normal	2.3

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Table 19

Baseline levels of IAA and 5-HIAA of normal individuals and patients with carcinoid tumor (14).

<u>Type</u>	<u>IAA (mg/24 hr)</u>	<u>5-HIAA (mg/24 hr)</u>	<u>IAA/5-HIAA</u>
Normal	4.71	3.07	1.5
Bronchial	0.6	755	0.0008
Ileal	17.63	381	0.046
Type IV	10.8	3.36	3.2

Fig. 40 Patient D.B. I urinary IAA/5-HIAA ratio determined by CIMS continuous ion monitoring of m/e 176 (IAA) and m/e 192 (5-HIAA).

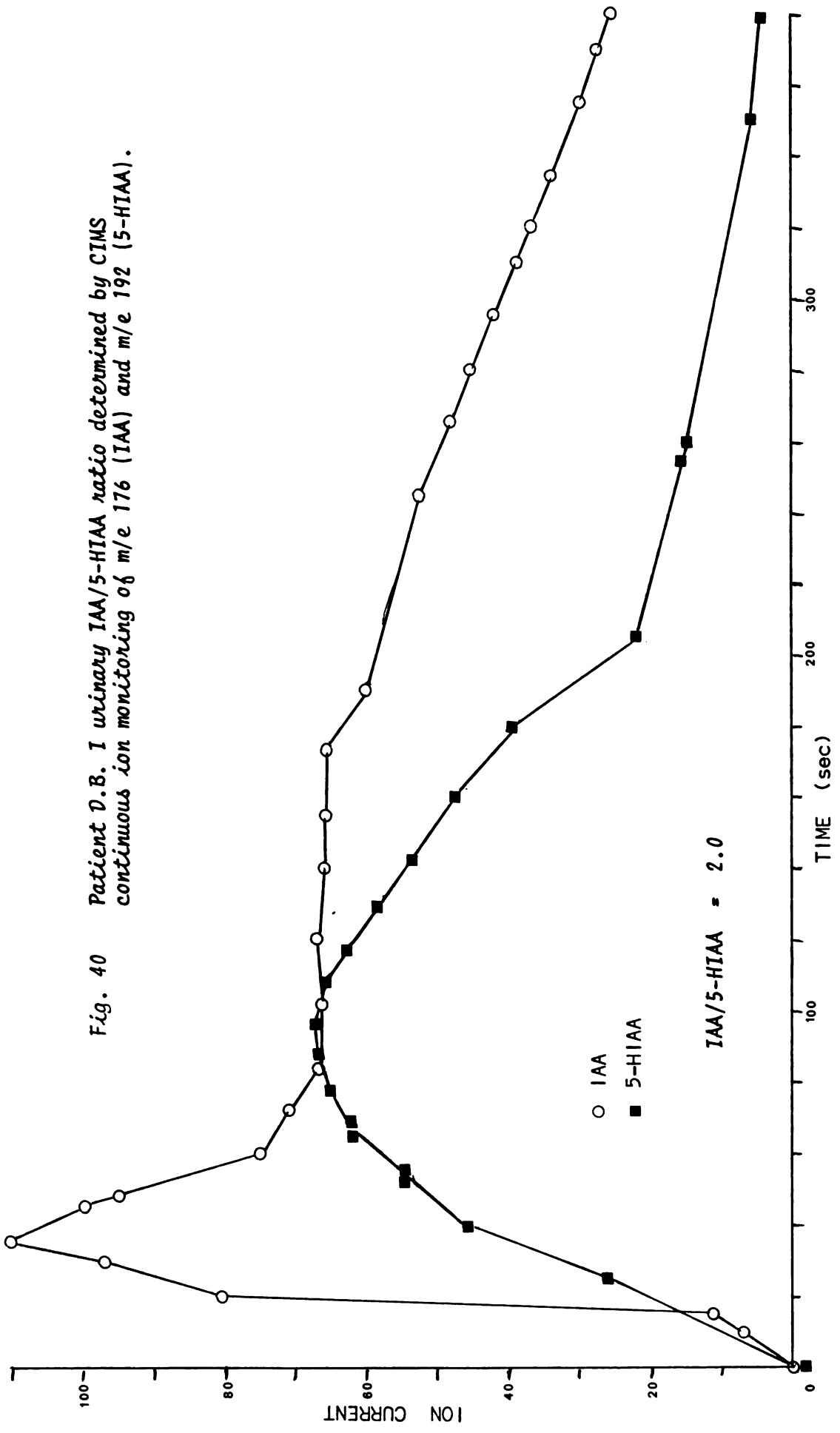
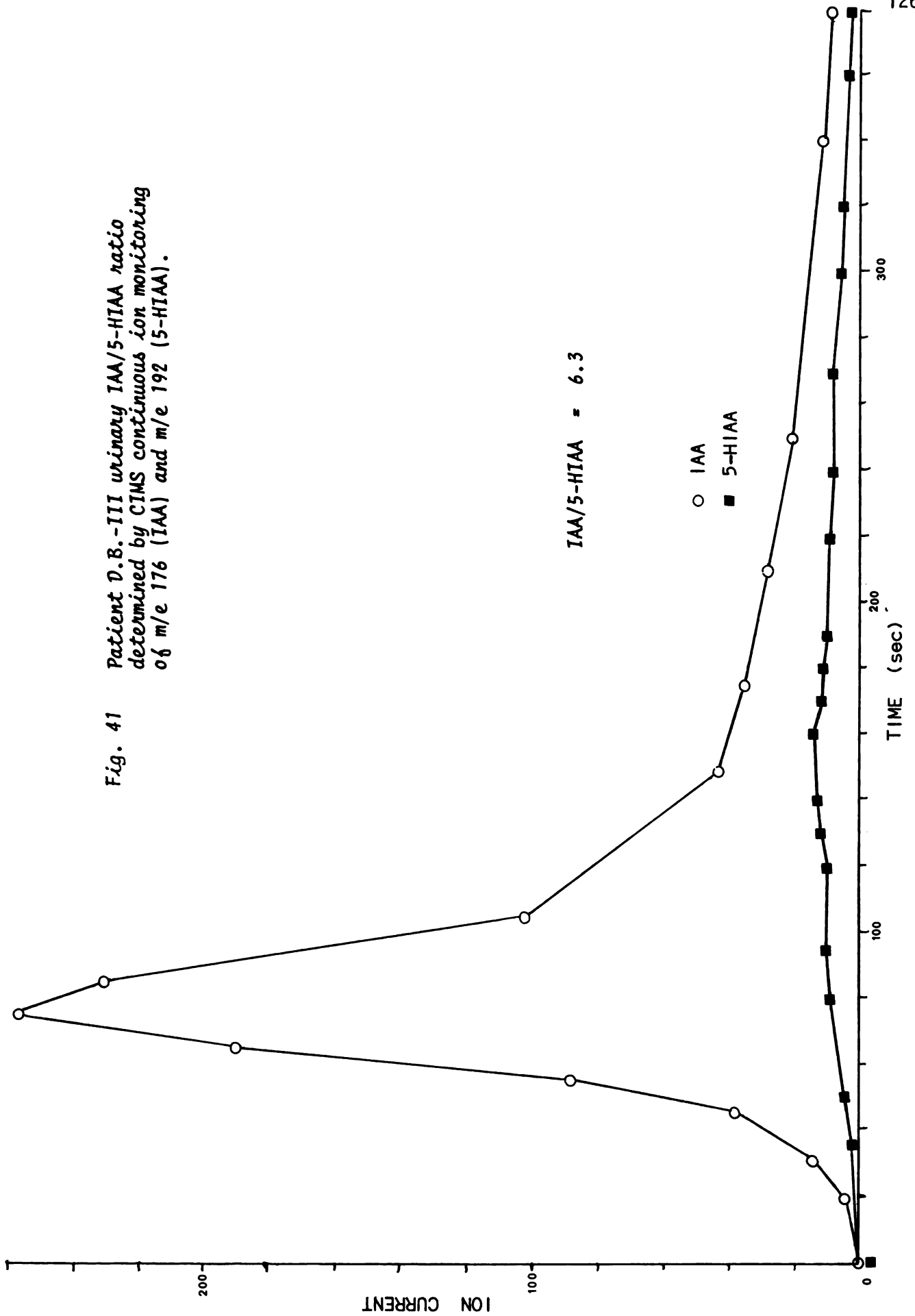


Fig. 41 Patient D.B.-111 urinary IAA/5-HIAA ratio determined by CIMS continuous ion monitoring of m/e 176 (IAA) and m/e 192 (5-HIAA).





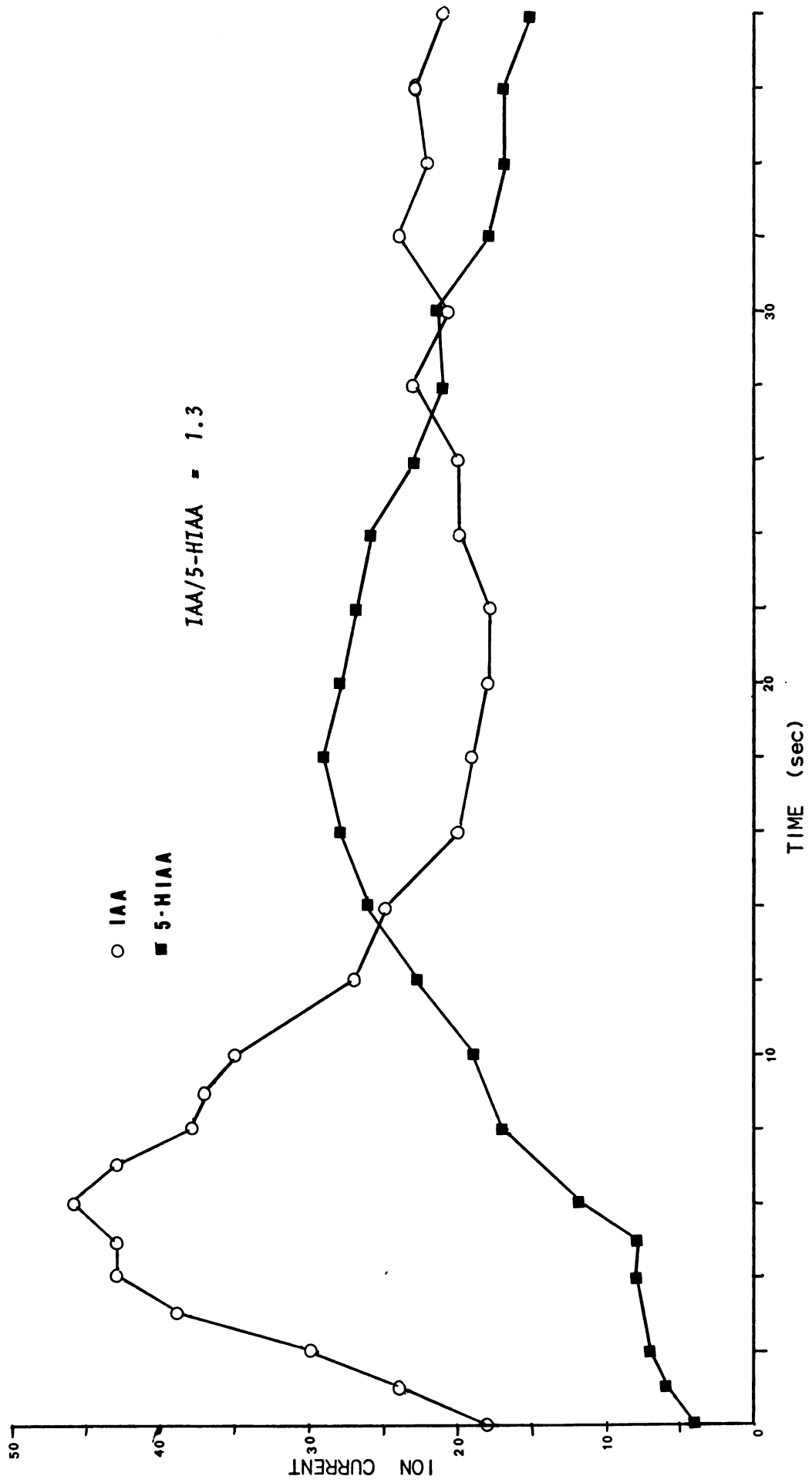
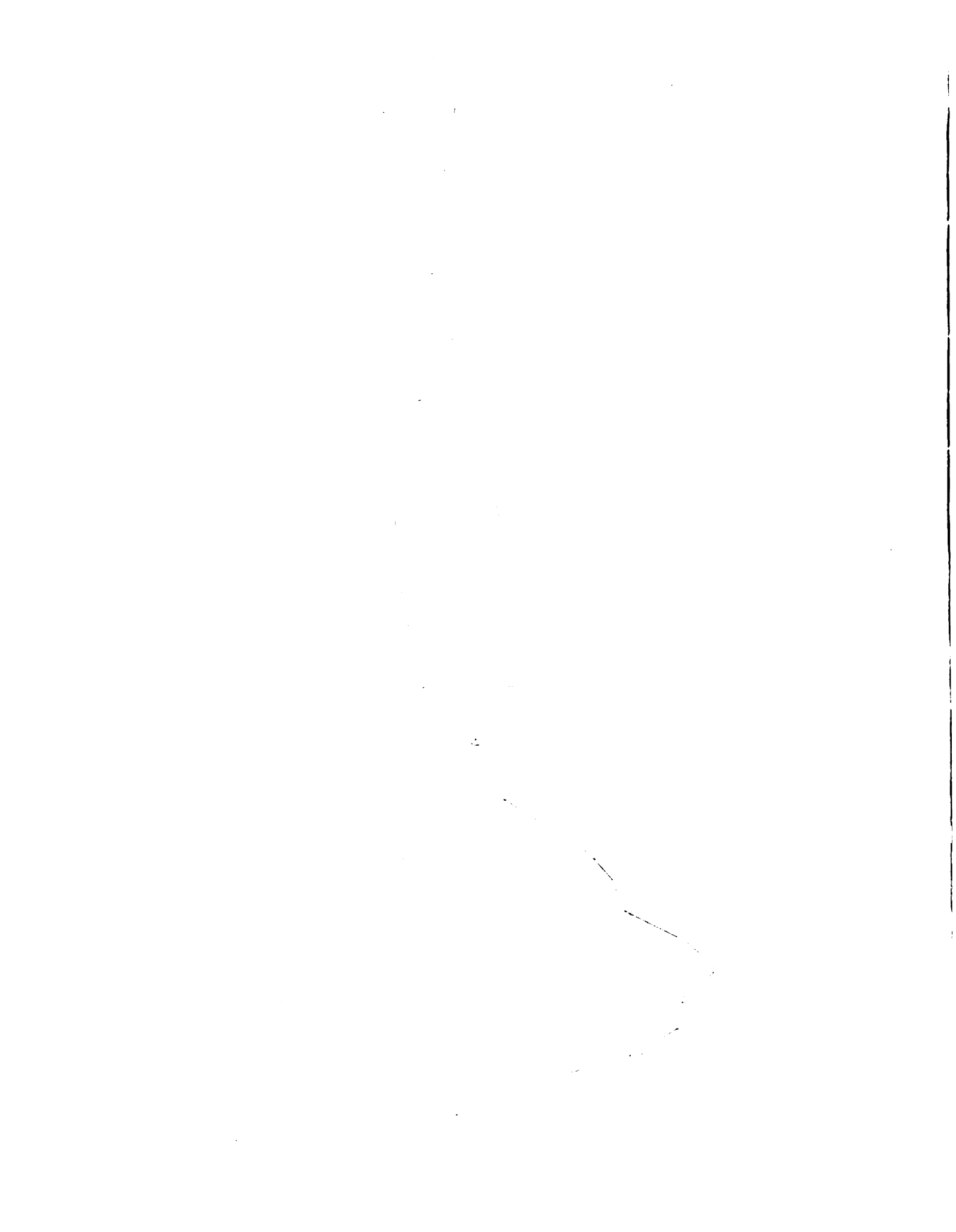
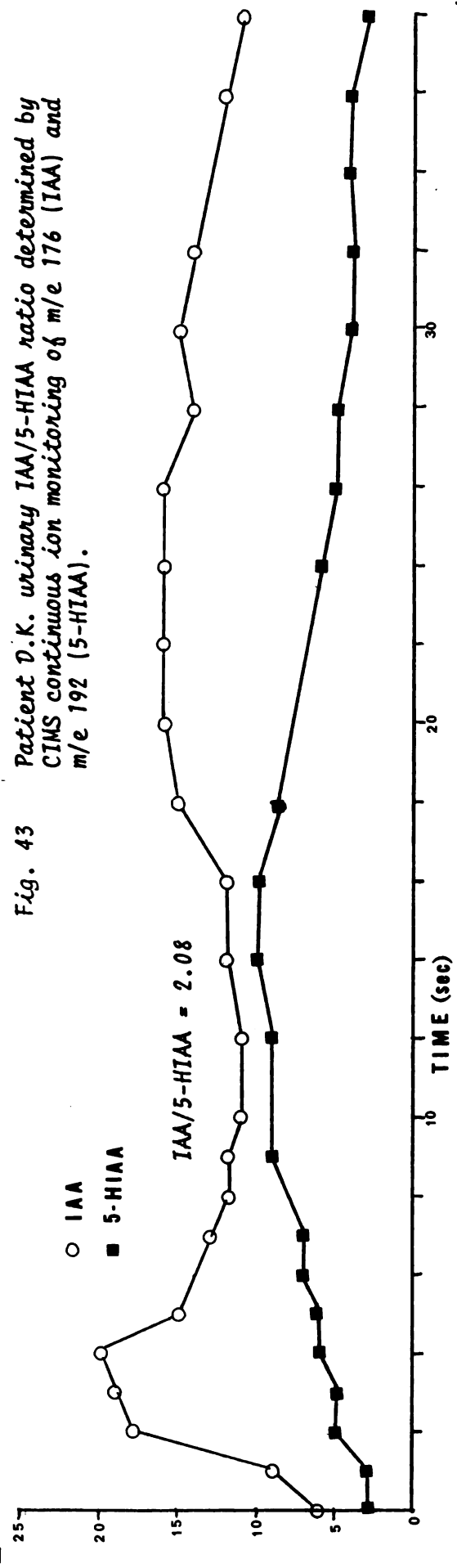
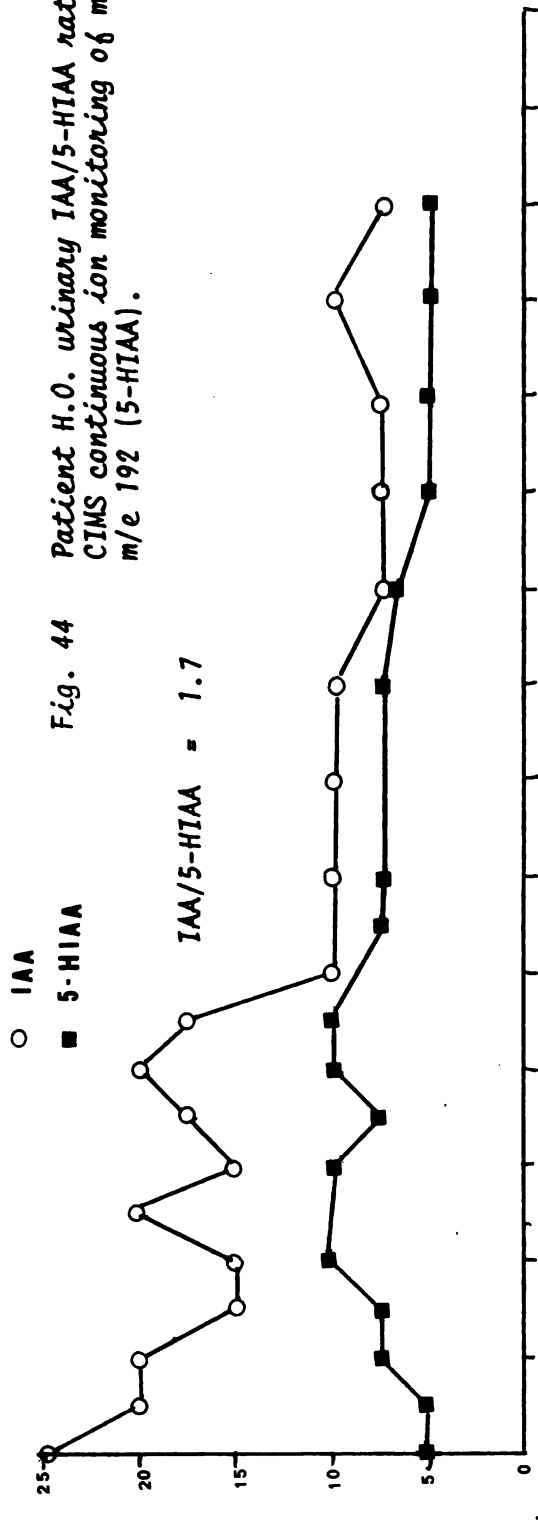


Fig. 42 Patient C.L. urinary IAA/5-HIAA ratio determined by CIMS continuous ion monitoring of m/e 176 (IAA) and m/e 192 (5-HIAA).





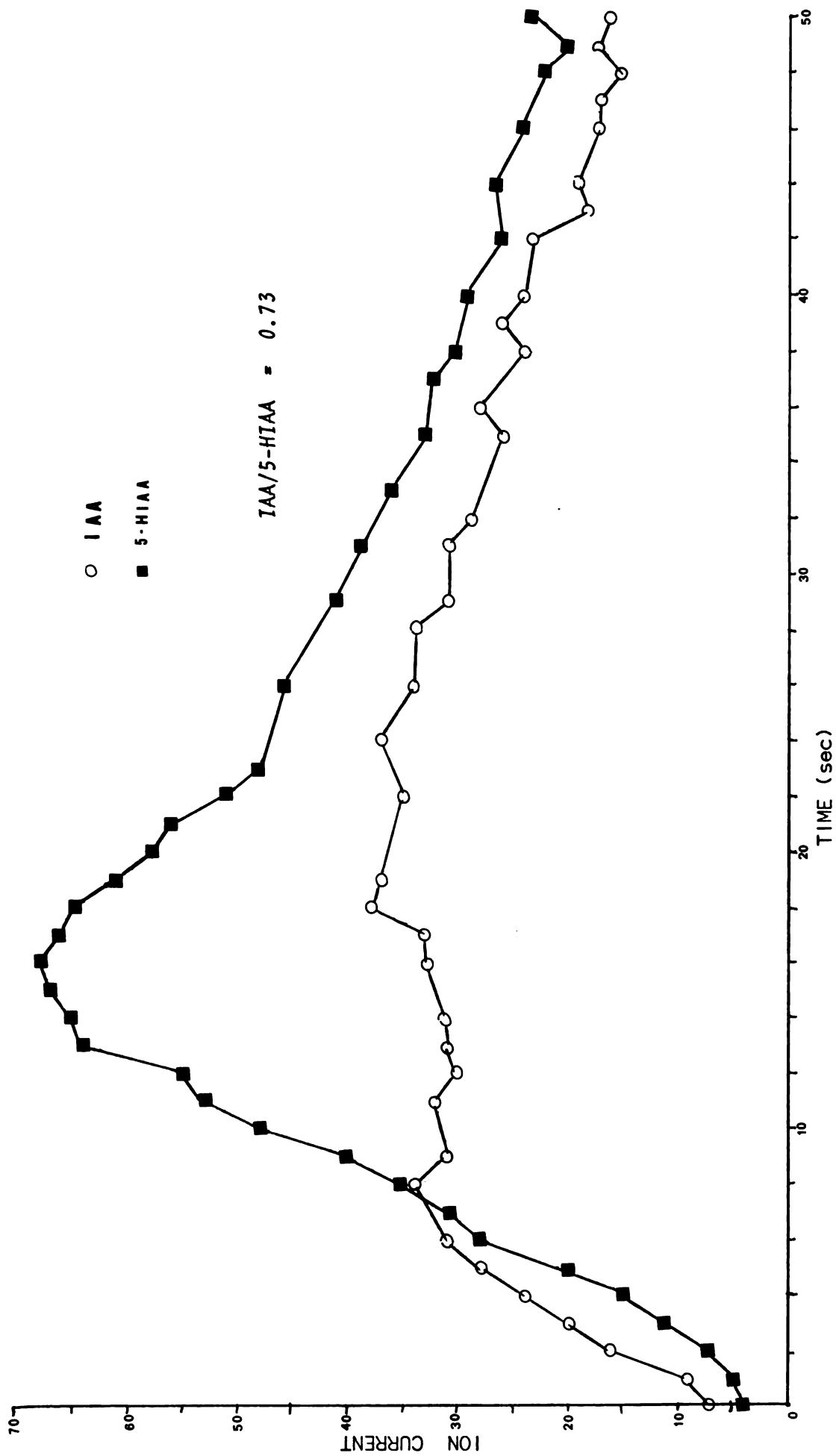
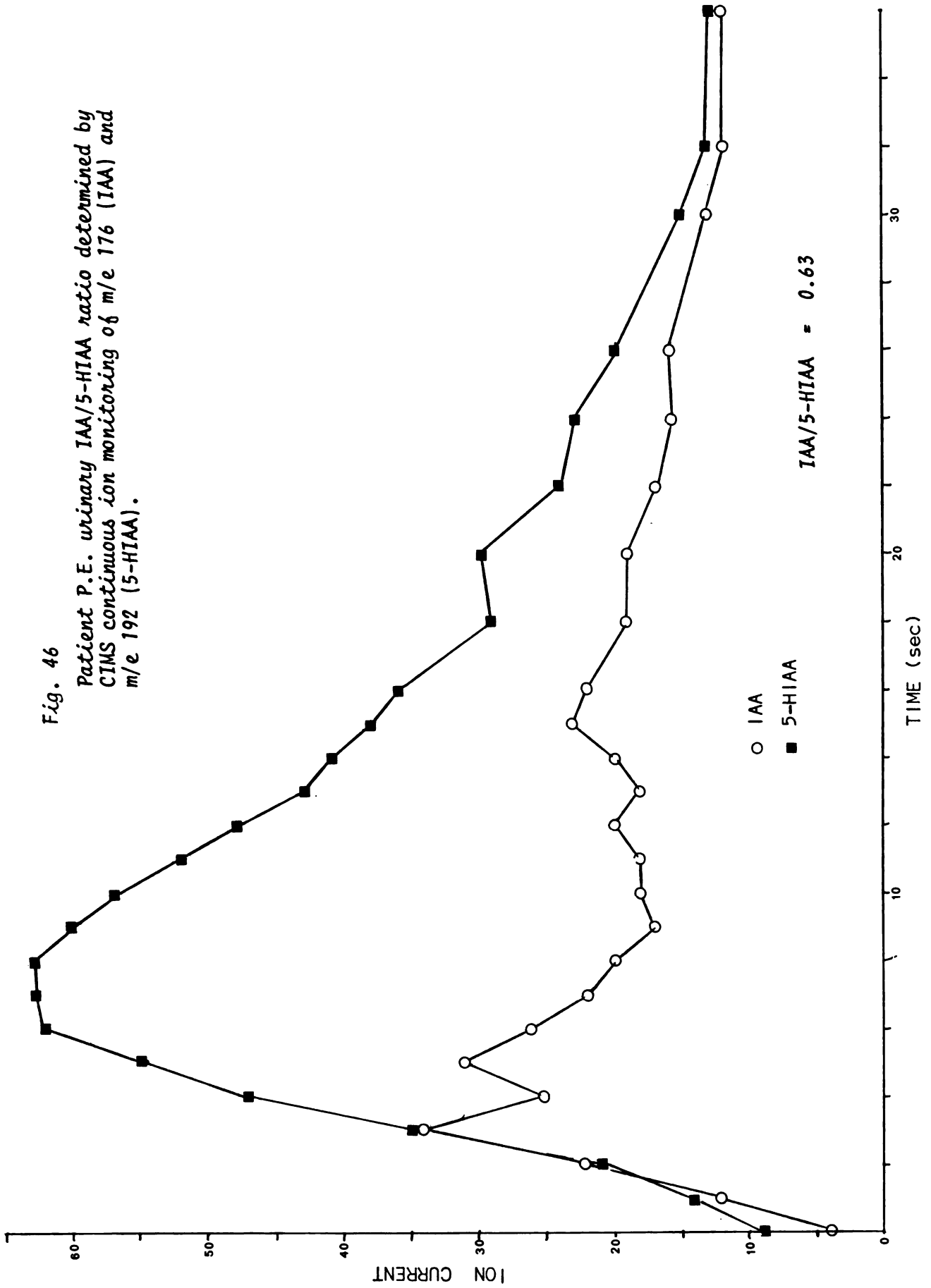


Fig. 45 Patient R.S. urinary IAA/5-HIAA ratio determined by CIMS continuous ion monitoring of m/e 176 (IAA) and m/e 192 (5-HIAA).

Fig. 46
 Patient P.E. urinary IAA/5-HIAA ratio determined by
 CIMS continuous ion monitoring of m/e 176 (IAA) and
 m/e 192 (5-HIAA).





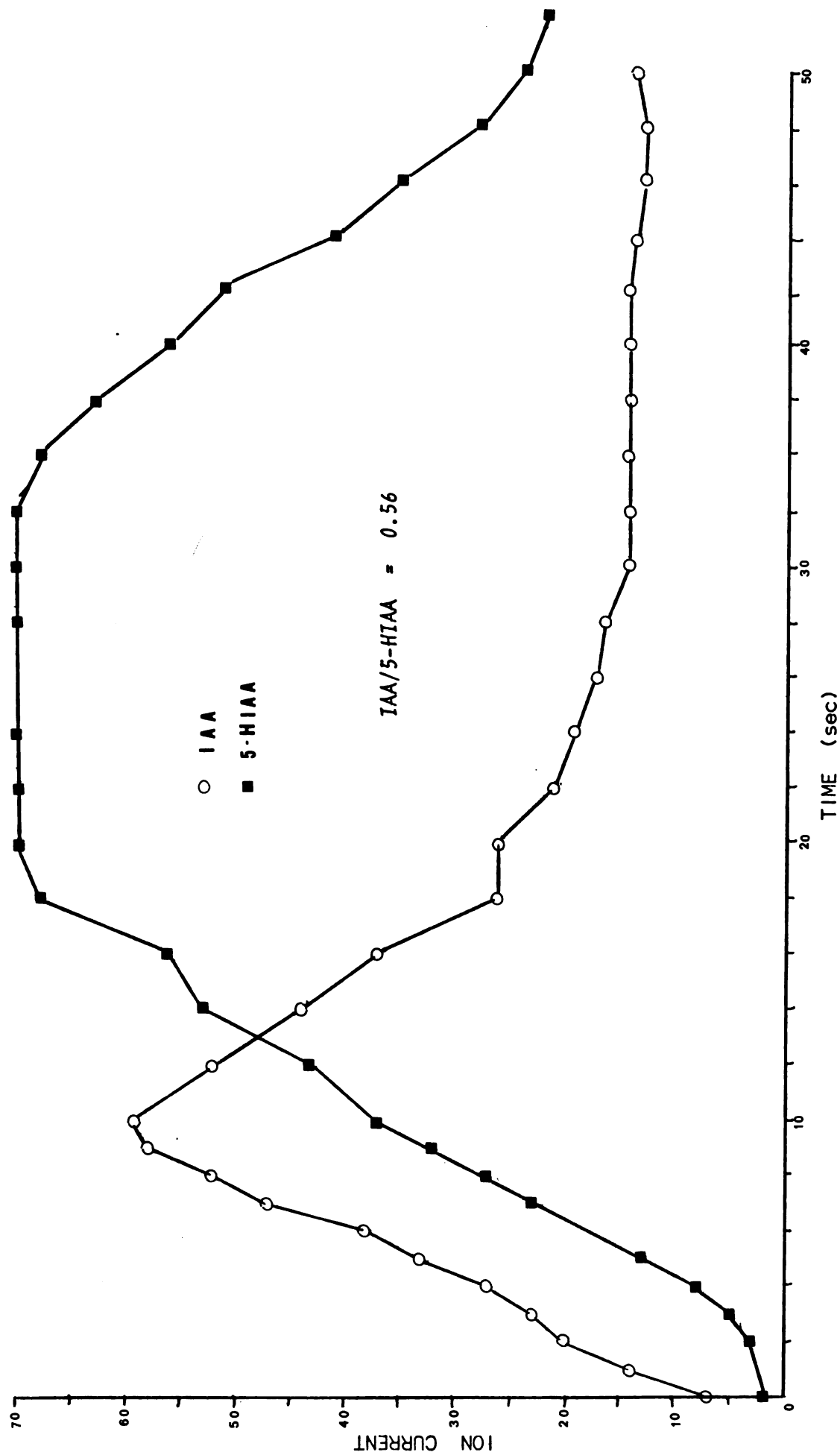


Fig. 47 Patient P.L. urinary IAA/5-HIAA ratio determined by CIMS continuous ion monitoring of m/e 176 (IAA) and m/e 192 (5-HIAA).



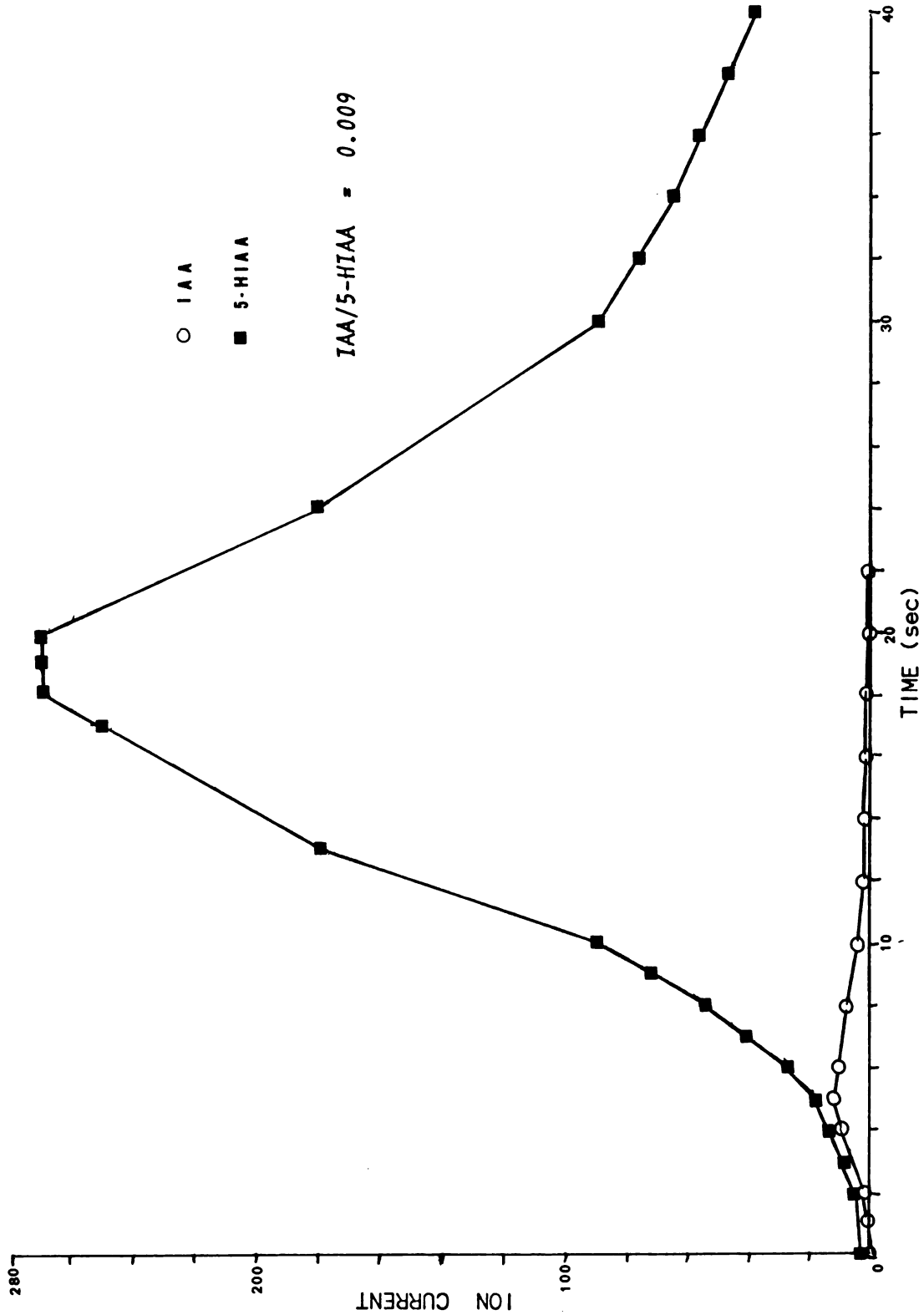
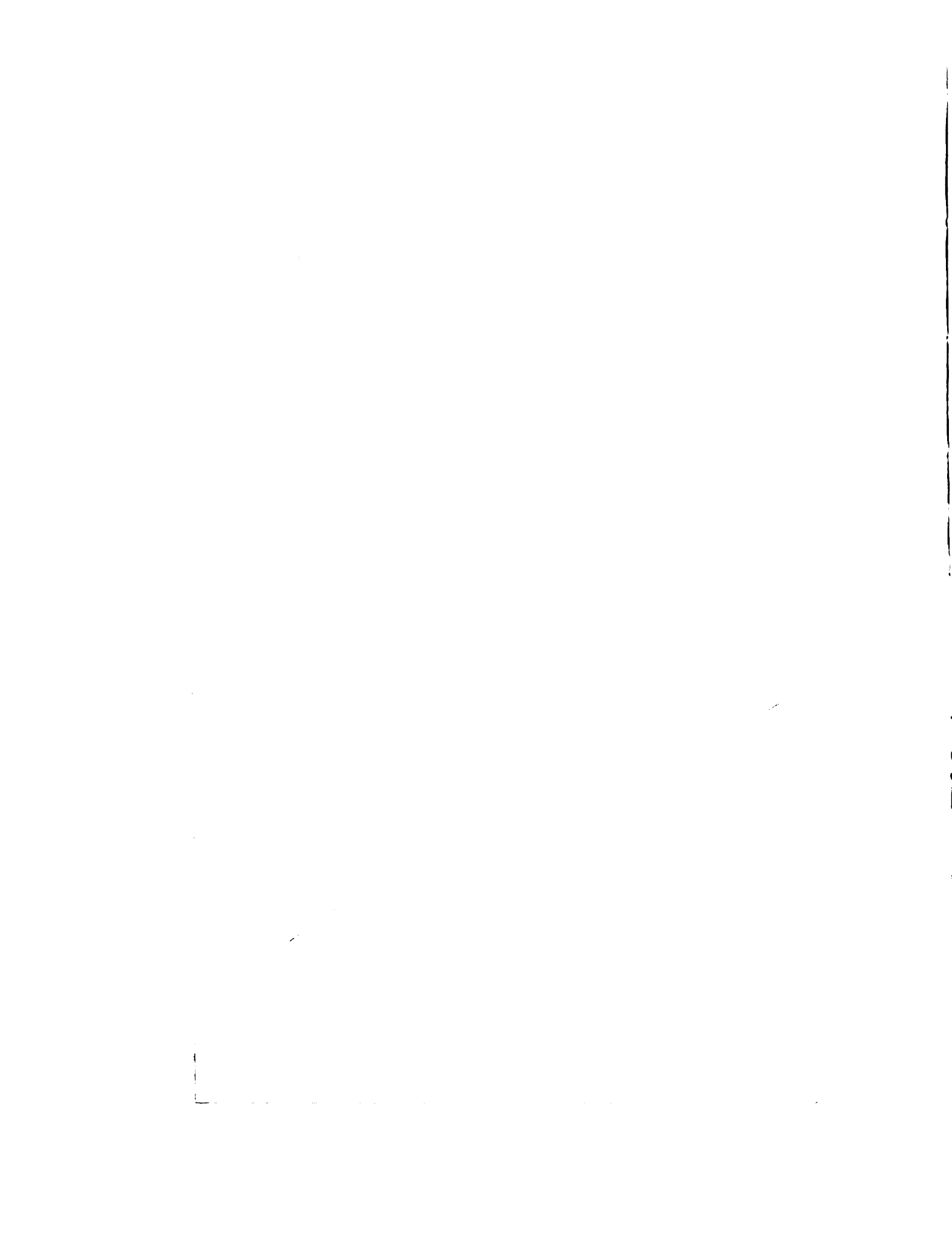


Fig. 48 Patient M.A. urinary IAA/5-HIAA ratio determined by CIMS continuous ion monitoring of m/e 176 (IAA) and m/e 192 (5-HIAA).



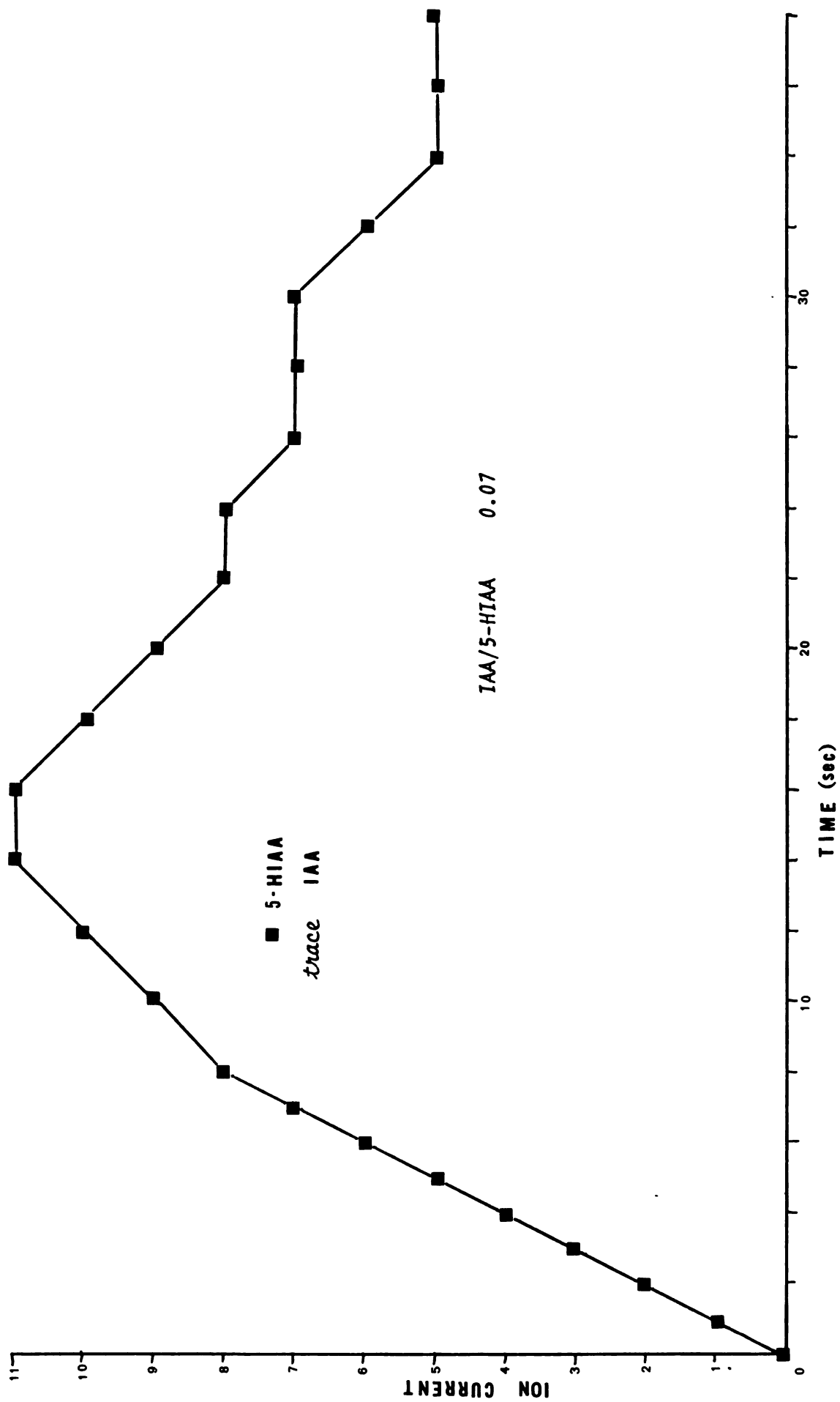
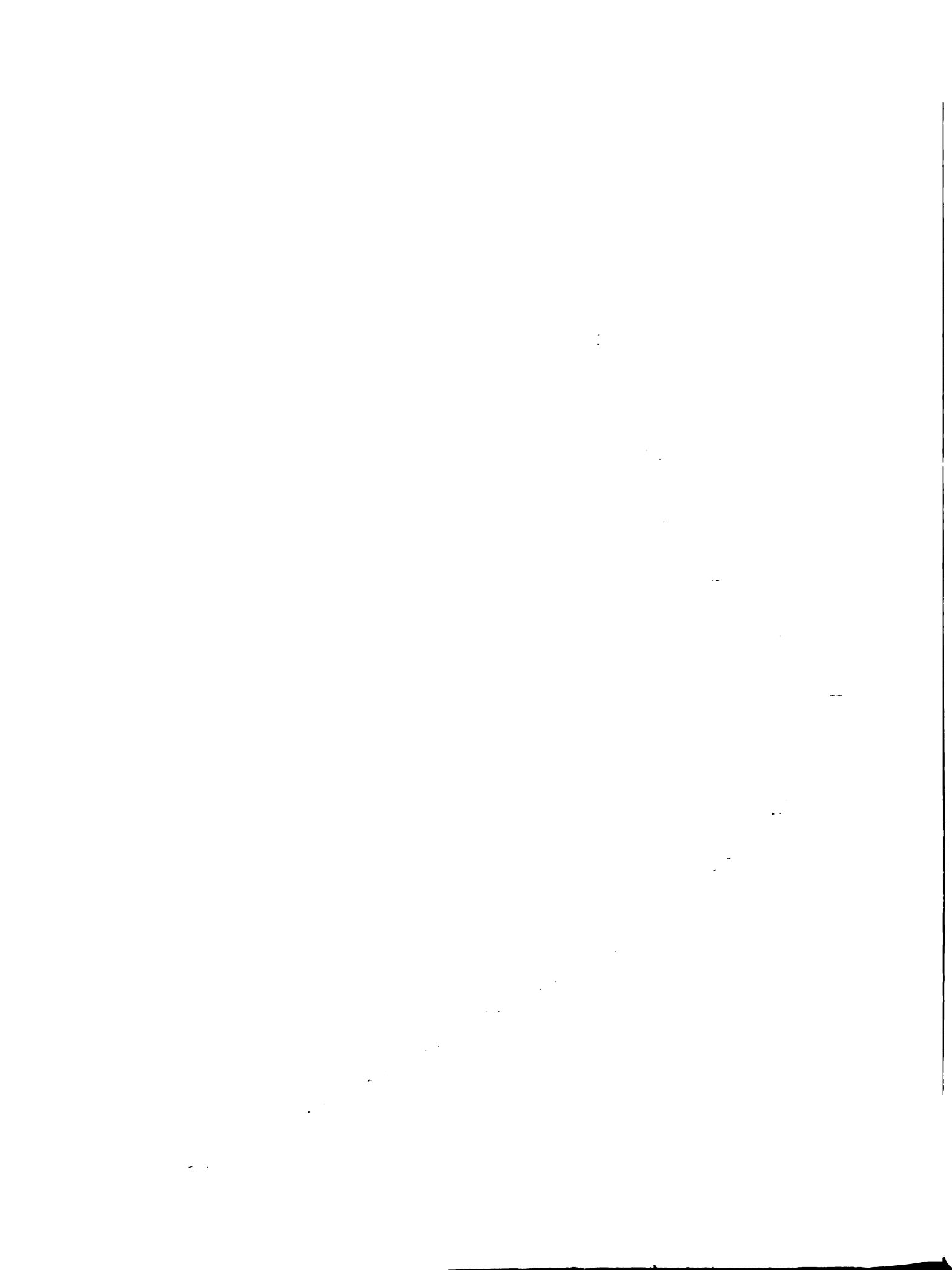


Fig. 49 Patient S.T. urinary IAA/5-HIAA ratio determined by CIMS continuous ion monitoring of m/e 176 (IAA) and m/e 192 (5-HIAA).



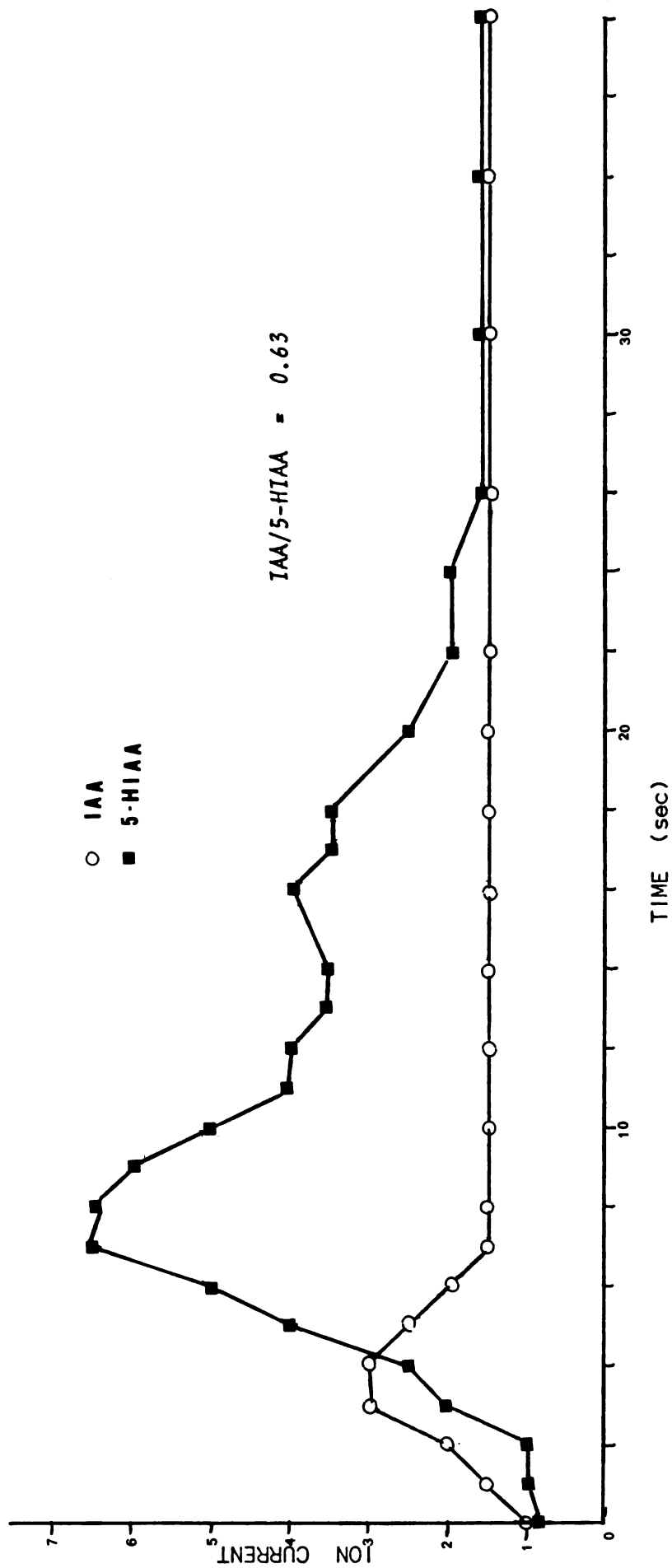
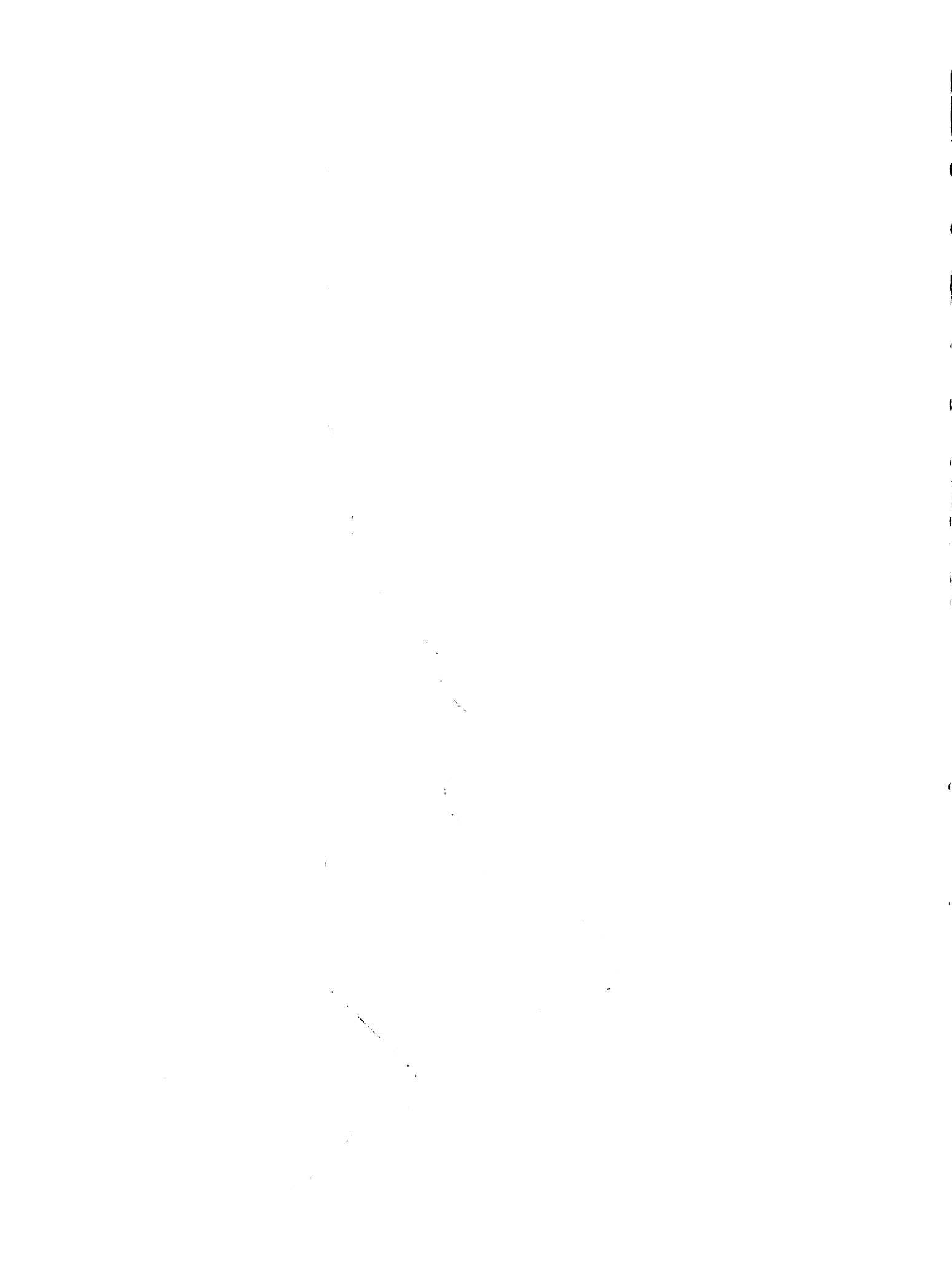


Fig. 50 Patient R.M. urinary IAA/5-HTAA ratio determined by CIMS continuous ion monitoring of m/e 176 (IAA) and m/e 192 (5-HTAA). Urine sample was collected prior to the administration of a tryptophan load.



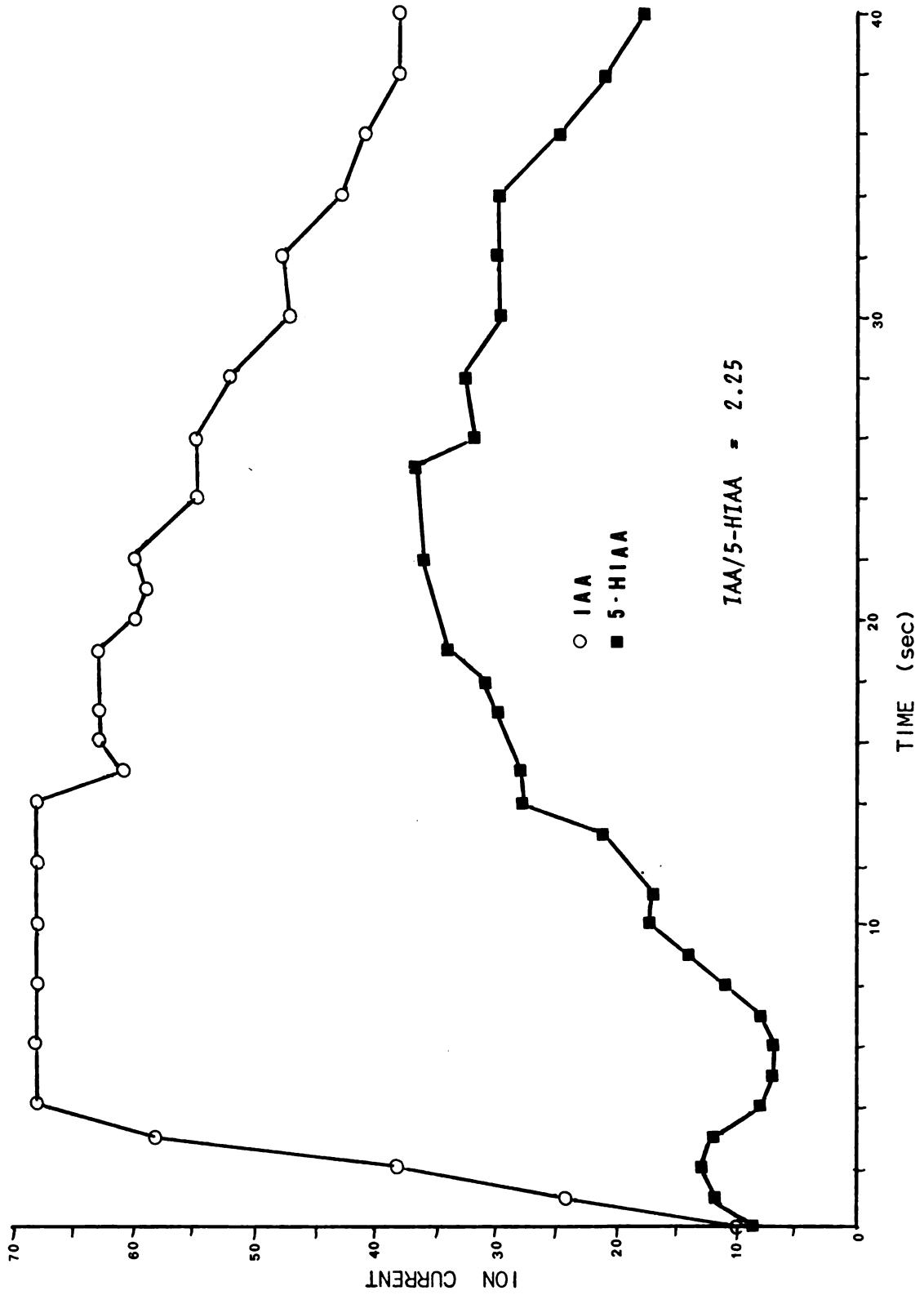


Fig. 51 Patient R.M. urinary IAA/5-HIAA ratio determined by CIMS continuous ion monitoring of m/e 176 (IAA) and m/e 192 (5-HIAA). Urine sample was collected 4 hr. after p.o. administration of L-tryptophan (100 mg/kg).



Table 20 show the results of C.I.M.S. analysis of two patient urines employing the internal standard assay procedure. Only two patient urines were available at this time. Therefore, no conclusion concerning quantities of metabolites found using this assay procedure can be made.

Table 20

Total amounts of urinary IAA, TOH and 5-HTOH excreted in 24 hr by patients as determined by CIMS internal standard assay.

<u>patient</u>	<u>compound</u>	<u>mg/24 hr</u>
D.B.-I	IAA	0.25
	TOH	(less than 17.1 μ g)
	5-HTOH	(less than 123 μ g)
B.A.	IAA	0.17
	TOH	(less than 12.5 μ g)
	5-HTOH	(less than 55 μ g)

1. The first part of the document discusses the importance of maintaining accurate records of all transactions and activities. It emphasizes that this is crucial for ensuring transparency and accountability in the organization's operations.

2. The second part of the document outlines the various methods and tools used to collect and analyze data. It highlights the need for consistent and reliable data collection processes to support effective decision-making.

3. The third part of the document focuses on the role of technology in data management and analysis. It discusses how modern software solutions can streamline data collection and provide valuable insights into organizational performance.

4. The fourth part of the document addresses the challenges associated with data collection and analysis. It identifies common pitfalls and offers strategies to overcome them, ensuring that the data remains accurate and useful.

5. The fifth part of the document discusses the importance of data security and privacy. It outlines best practices for protecting sensitive information and ensuring compliance with relevant regulations.

6. The sixth part of the document explores the role of data in strategic planning and decision-making. It illustrates how data-driven insights can inform key business decisions and drive long-term success.

7. The seventh part of the document discusses the importance of data literacy and training. It emphasizes that all employees should have the skills and knowledge to effectively use data in their work.

8. The eighth part of the document discusses the importance of data governance. It outlines the policies and procedures needed to ensure that data is managed consistently and responsibly across the organization.

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10. The tenth part of the document discusses the importance of data visualization. It explains how visual representations of data can make complex information easier to understand and communicate to stakeholders.

11. The eleventh part of the document discusses the importance of data archiving and backup. It outlines the best practices for ensuring that data is preserved and can be recovered in the event of a disaster.

12. The twelfth part of the document discusses the importance of data ethics. It emphasizes the need to use data responsibly and to be transparent about how it is collected and used.

13. The thirteenth part of the document discusses the importance of data innovation. It highlights the need to explore new ways of using data to drive business growth and create competitive advantages.

14. The fourteenth part of the document discusses the importance of data collaboration. It emphasizes the need for different departments and teams to share data and work together to solve complex problems.

15. The fifteenth part of the document discusses the importance of data reporting. It outlines the best practices for creating clear and concise reports that provide actionable insights to decision-makers.

16. The sixteenth part of the document discusses the importance of data monitoring and evaluation. It emphasizes the need to track key performance indicators and evaluate the impact of data-driven initiatives over time.

17. The seventeenth part of the document discusses the importance of data communication. It highlights the need to effectively communicate data findings to all relevant stakeholders in a clear and compelling way.

18. The eighteenth part of the document discusses the importance of data culture. It emphasizes the need to foster a culture where data is valued and used to drive decision-making at all levels of the organization.

19. The nineteenth part of the document discusses the importance of data future. It explores emerging trends and technologies that will shape the future of data management and analysis.

Part IV

C. In Vitro Experiments

In Vitro Tumor Tissue Incubations With ^{14}C -L-Tryptophan

Introduction

An attempt was made to study the in vitro metabolism of tryptophan by tumor tissue. Tissue is best obtained at biopsy but the rarity of surgery associated with carcinoid tumors combined with the modern technique of needle biopsy, which removes only a few milligrams of tissue, made it impossible to obtain sufficient tissue by this means. The only remaining way to obtain samples was at autopsy and the chance arose only once in six years.

Previous attempts to demonstrate in vitro metabolism of tryptophan by carcinoid tumors have consisted of tissue slice incubations from metastatic tissue obtained at autopsy or assay of tryptophan hydroxylase activity (13,96). Demonstration of in vitro metabolism of tryptophan carcinoid tumor tissue remains an important unresolved determinant in assigning the tumor as the tissue responsible for in vivo production of tryptophan metabolites. My experiments were conducted with liver metastatic tumor obtained at autopsy 15 hr post mortum.

Methods of Procedure

A 23 yr old male with biopsy proven carcinoid tumor (urinary 5-HIAA levels 220-740 mg/24 hr) died after experiencing a hypotensive crises with a total body flush. At 15 hr post mortum, an autopsy was performed. Using sterile techniques, a 10 gram piece of tumor tissue from the liver was removed and placed in cold 0.06 M phosphate buffer, pH 7.4. Three homogenates were prepared: 15% (weight/volume), 10% (w/v) and 5% (w/v). The homogenates were incubated with 3-¹⁴C-L-tryptophan (New England Nuclear), 23.3 µg/3.33 µCi, at a concentration of approximately 155 µg tryptophan per gram tissue. Controls consisted of incubations with boiled homogenate and no homogenate present. Incubates were prepared according to table 21 and were conducted simultaneously at 37°C in air for 2 hr. The incubates were then stored frozen for later analysis. Analysis consisted of thawing, addition of acetone to precipitate the protein and high speed liquid chromatographic analysis of the supernatant. The protein precipitate was weighed and radioactivity associated with the protein counted by liquid scintillation after digestion by Protosol (New England Nuclear).

The liquid chromatography (L.C.) apparatus was equipped with a dual wave length uv detector (254 and 280 nm). All L.C. procedures were conducted using a Zipa strong cation exchange column eluted with 4N or 2N acetic acid at a rate of 0.4 ml/min or 0.2 ml/min. Liquid scintillation counting was performed by a Hewlett Packard Tri-Carb Liquid Scintillation Counter. Efficiency was determined by external standard.

The first part of the document discusses the importance of maintaining accurate records of all transactions and activities. It emphasizes that this is crucial for ensuring transparency and accountability in the organization's operations.

Furthermore, it highlights the need for regular audits and reviews to identify any discrepancies or areas for improvement. This process should be conducted by an independent body to ensure objectivity and fairness.

In addition, the document outlines the responsibilities of various stakeholders, including management, employees, and external auditors. Each party has a role to play in ensuring the integrity and reliability of the financial information provided.

It also addresses the challenges associated with data collection and analysis, particularly in large-scale organizations. Advanced technologies and software solutions are recommended to streamline these processes and reduce the risk of human error.

The document concludes by reiterating the commitment to high standards of ethical conduct and professional behavior. It encourages all members of the organization to uphold these values and contribute to the overall success and reputation of the institution.

Finally, it provides a summary of the key findings and recommendations. These include the need for enhanced training, improved communication channels, and the implementation of robust internal controls to prevent future issues.

The document is intended to serve as a guide for all stakeholders involved in the organization's financial and operational activities. It is subject to periodic updates to reflect changes in regulations and best practices.

For more information or to request a copy of this document, please contact the relevant department. We are committed to providing clear and accessible information to all our stakeholders.

Table 21

Composition of metastatic tumor tissue incubations with 3-¹⁴C-L-tryptophan. All incubations were conducted simultaneously at 37°C in air for 2 hr.

<u>co-factors</u>	<u>final conc.</u>
0.2 ml ATP	0.01M
0.1 ml NADP	0.003M
0.1 ml NAD	0.003M
0.1 ml Nicotinamide	0.6M
0.2 ml G-6-P	0.006M
0.1 ml MgCl	0.1M
0.1 ml KCl	0.2M

Total 0.9 ml

Incubate (total volume 2.0 ml)

- 1-A 1.0 ml 15% (w/v) homogenate, 0.1 ml ¹⁴C-TP, 0.9 ml co-factors
- 1-B 1.0 ml 15% homogenate boiled for 20 minutes, ¹⁴C-TP and co-factors
- 2-A 10% homogenate, ¹⁴C-TP, co-factors
- 3-A 5% homogenate, ¹⁴C-TP, co-factors
- 4-A 1.0 ml distilled water, ¹⁴C-TP, co-factors
- 5-A 10% homogenate, ¹⁴C-TP, co-factors, 0.05 M 2-mercaptoethanol
- 5-B 10% homogenate boiled for 20 minutes, ¹⁴C-TP, co-factors, and 0.05 M 2-mercaptoethanol
- 6-A 10 ml 15% homogenate, 1.0 ml ¹⁴C-TP, 9.0 ml total volume of co-factors (10x all values - total volume 20 ml)

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Results and Discussion

The primary use of L.C. was to determine if in vitro metabolism of tryptophan occurred. As seen in fig. 52 there are significant differences between the experimental incubate 1-A and the control, 1-B. Tryptophan elutes at 10-15 minutes when the column is eluted with 4.0 N HOAc at a flow of 0.4 ml/min which corresponds to fractions 4-6. The fact that a large portion of the radioactivity of incubate 1-A elutes within the first 7 minutes is important in light of the fact that deaminated tryptophan metabolites elute very quickly, usually within the first 5 minutes, independent of acetic acid concentration. However, over-loading an L.C. column causes quicker elution of a compound. Noting fig. 52 it appears as if the graph of 1-A is shifted to the left of 1-B but is otherwise very similar. Since 1-B was chromatographed first followed by 1-A, it could be that un-eluted material (co-factors, etc.) remained on the column thus taking up active sites on the Zipax. Subsequent elution of the next fraction would result in a shorter retention time. To test this idea, incubates 6-A and 4-A were eluted in reverse order from that of 1-A and 1-B, that is experimental (6-A) first, followed by the control (4-A) and the concentration of acetic acid was reduced from 4.0 N to 2.0 N and the flow from 0.4 to 0.2 ml/min. This increased the retention time for tryptophan to 25-35 min. The results can be seen in figs. 53 and 54a,b. "Cold" tryptophan was added to incubates 6-A and 4-A in order to facilitate u.v. detection of tryptophan. It was perhaps too large an amount of cold tryptophan added to 4-A that caused the elution of tryptophan to occur at fraction 8 (fig. 54b) rather than at fraction 13

as in incubate 6-A. Nevertheless, 46.8% of the radioactivity in 6-A was eluted in the first five fractions.

Yet, it was still possible that overloading the column caused a large amount of radioactivity of elute in fractions 2 through 5 of incubate 6-A. If fractions 2-5 contained radio-labeled compounds that were not tryptophan, one would expect these fractions to maintain their chromatographic character when chromatographed separately. Therefore, incubate 6-A was rechromatographed (fig. 55) and fraction 2 which eluted between 2 and 6 minutes was collected. Fraction 2 had a total volume of 1.6 ml. which was concentrated to dryness, redissolved in 0.2 ml H₂O and 0.05 ml of this solution was applied to the column. Figure 56 shows the results of scintillation counting. It is clear that fraction 2 did not maintain its chromatographic integrity. Instead, the compound(s) in fraction 2 eluted at the same time as the substrate, tryptophan (24 min.). This seemed to support the idea that the early elution of radioactivity was an artifact of the L.C. system and procedure and not an indication of metabolism by the tumor tissue. In addition, chemical ionization mass spectral analysis of incubation 6-A could not demonstrate the presence of any metabolites of tryptophan.

Additional experiments with incubates 1-6 were not conducted. It was felt that further experimentation with tumor tissue obtained 15 hr post mortum and therefore of questionable viability would be wasted effort.

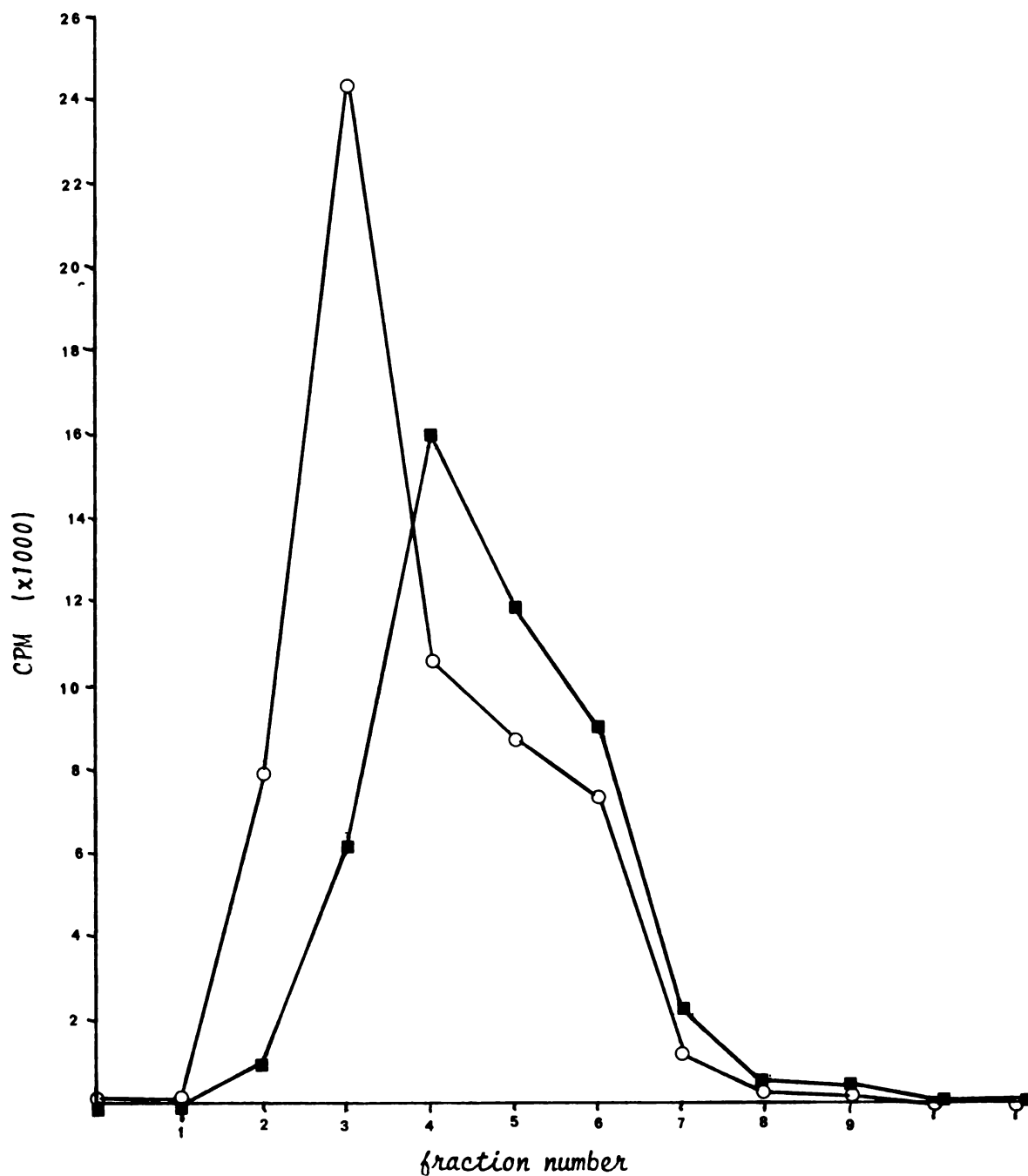
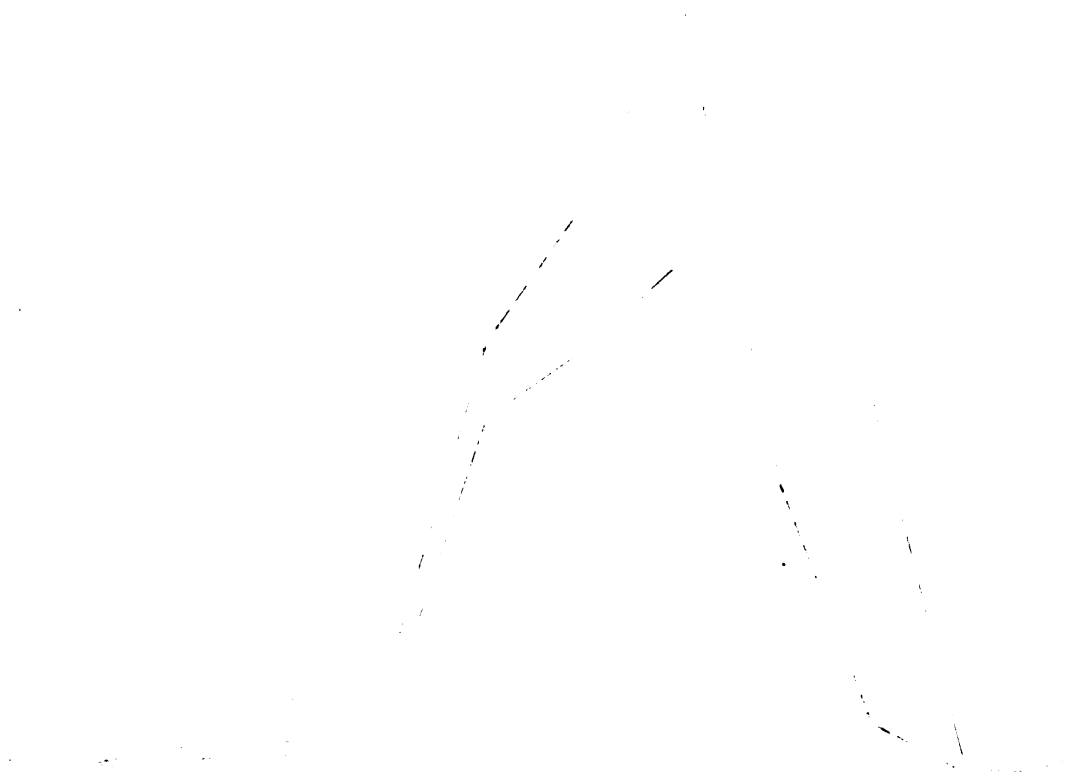


Fig. 52

Radioactivity of carcinoid tumor incubates 1-A \circ and 1-B \blacksquare eluted from a liquid chromatography cation exchange column. (Solvent: 4.0 N acetic acid; Flow: 0.4 ml/min)



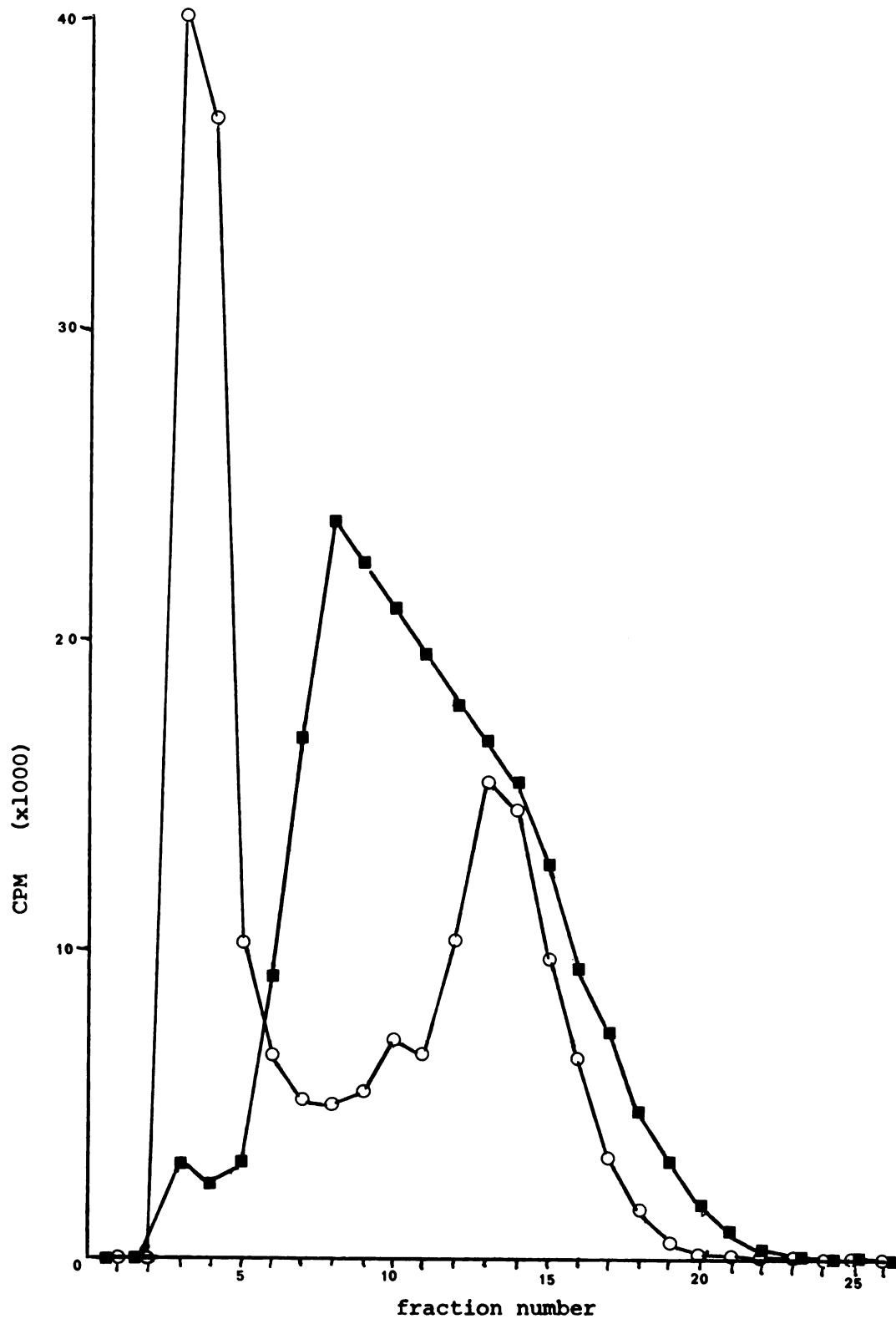


Fig. 53 Radioactivity of carcinoid tumor incubates 6-A \circ and 4-A \blacksquare eluted from a liquid chromatography cation exchange column. (Solvent: 2.0 N acetic acid; Flow: 0.2 ml/min)

Fig. 54a

Liquid chromatography of incubate 6-A
of L-TP with carcinoid tumor homogenate.
UV detector at 254 nm :..... and at
280 nm ——— Column eluted with 2.0
N acetic acid at 0.2 ml/min.

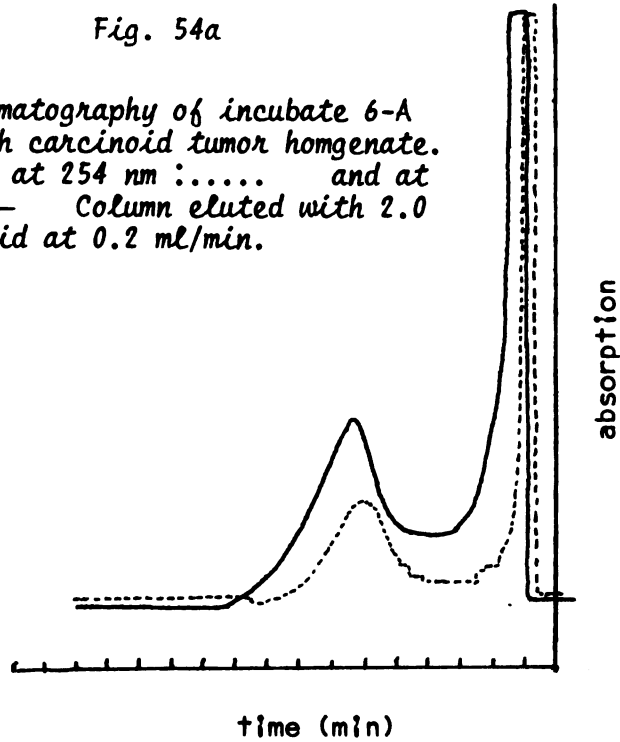
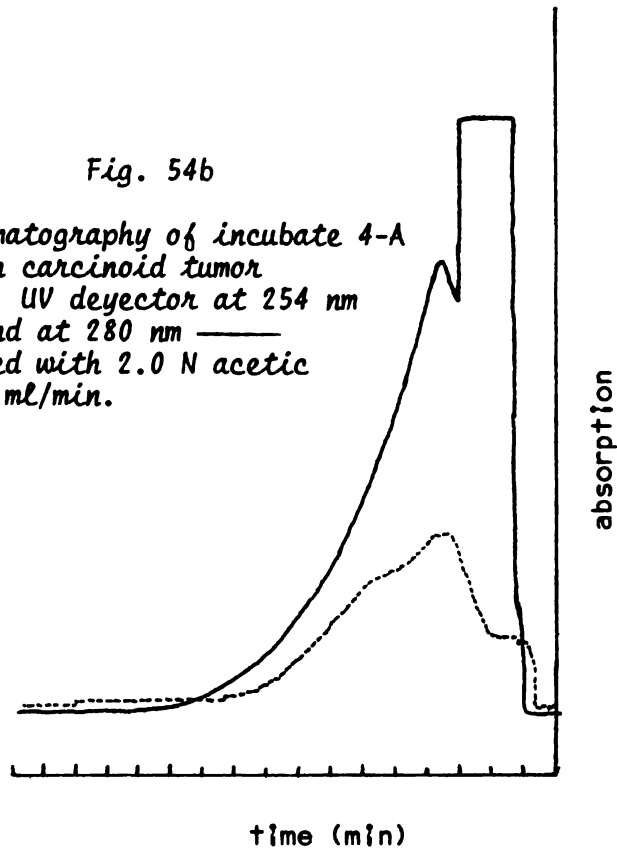


Fig. 54b

Liquid chromatography of incubate 4-A
of L-TP with carcinoid tumor
homogenate. UV dejector at 254 nm
.... and at 280 nm ———
Column eluted with 2.0 N acetic
acid at 0.2 ml/min.



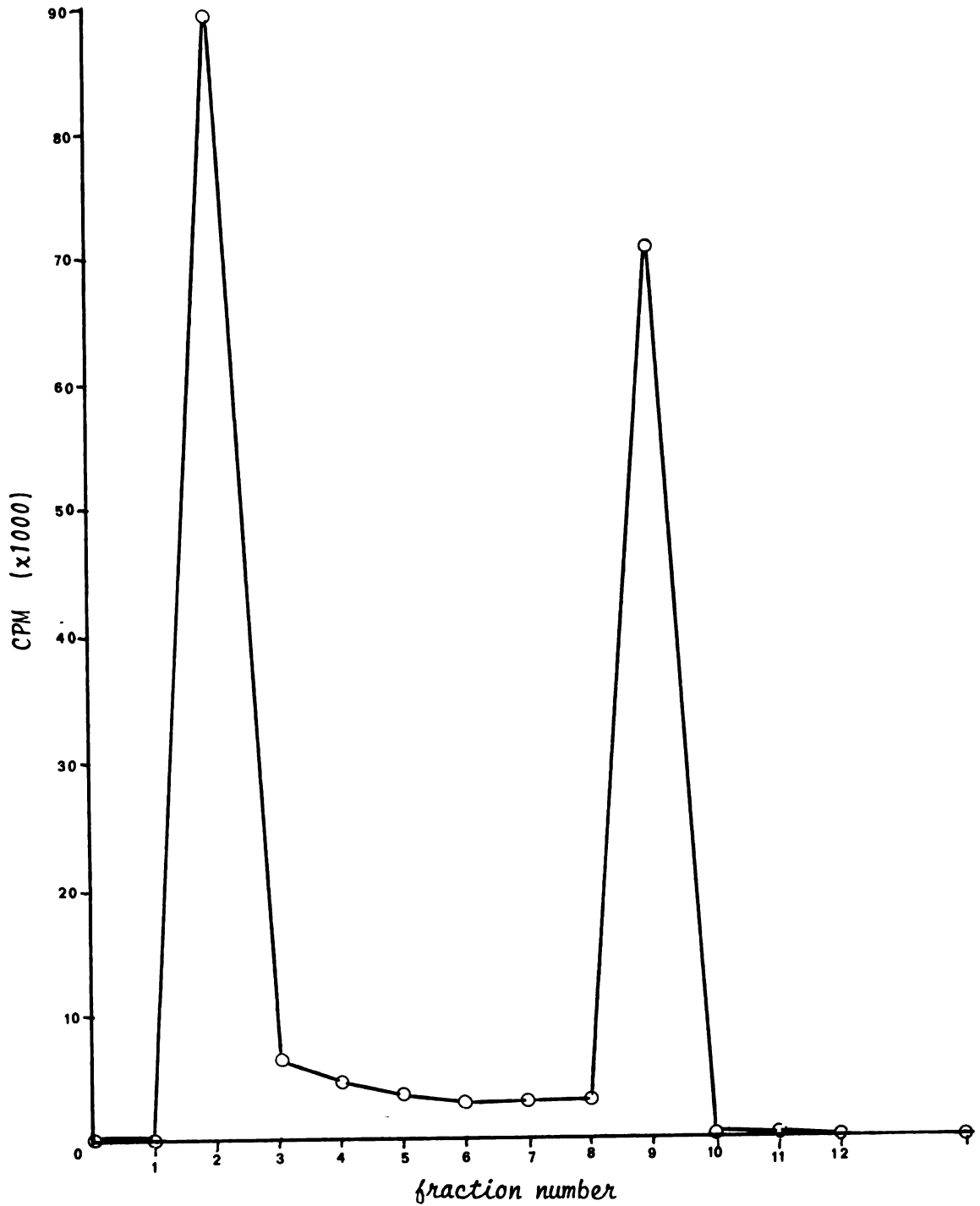


Fig. 55 Radioactivity of carcinoid tumor incubate 6-A eluted from a liquid chromatography cation exchange column. (Solvent: 4.0 N acetic acid; Flow: 0.2 ml/min)

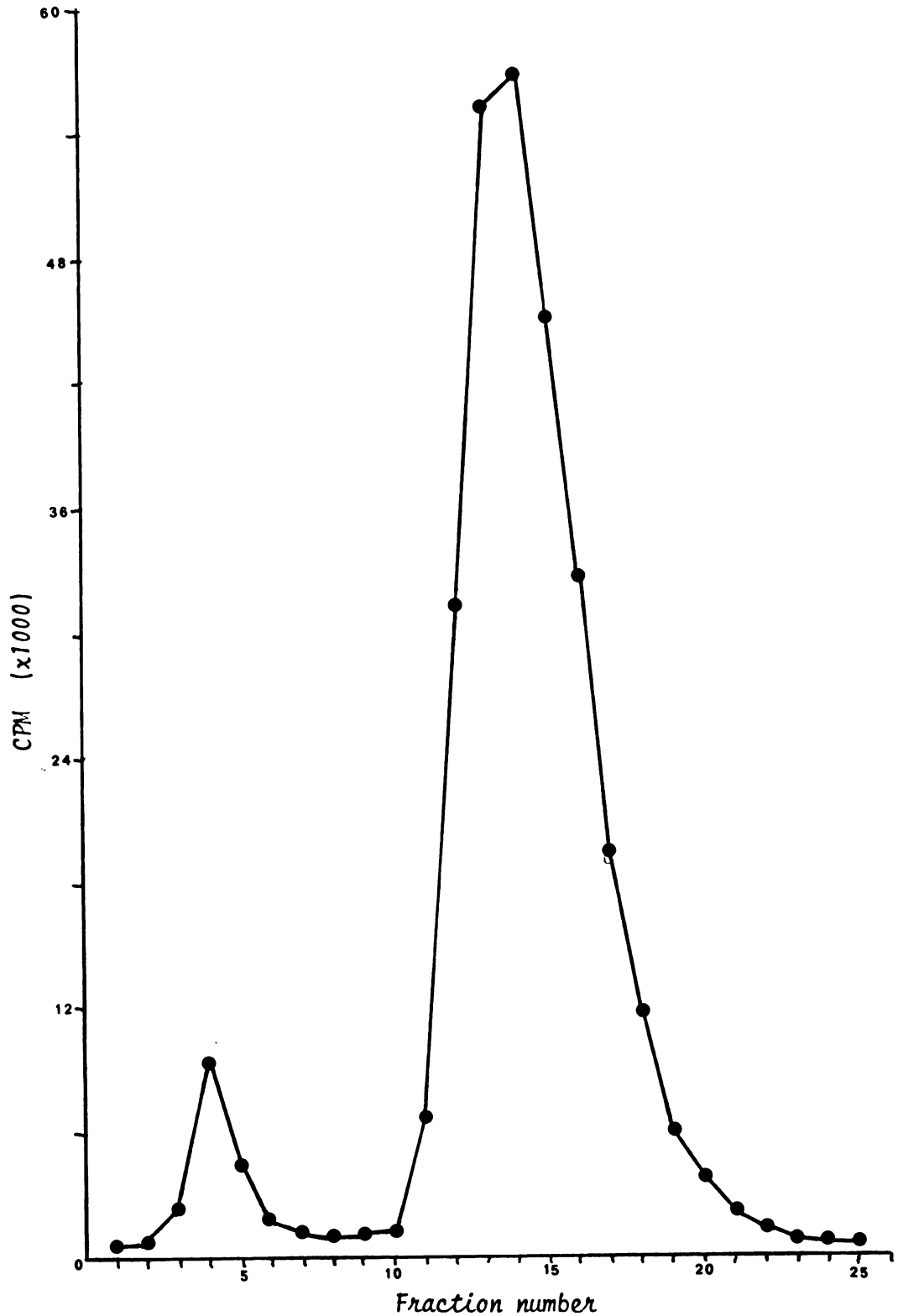


Fig. 56 Radioactivity of fraction 2 of carcinoid tumor incubate 6-A eluted from a liquid chromatography cation exchange column. (Solvent: 4.0 N acetic acid; Flow: 0.2 ml/min)

Part IV

D. Pharmacological Activity of Indoleacrylic Acid

Indoleacrylic Acid: Possible Hypoglycemic Activity

The variety of symptoms in patients with the carcinoid syndrome has raised questions about the role of the various metabolites of tryptophan in the clinical syndrome. One of the more bizarre symptoms presented by patients with carcinoid tumor is a craving of sweets, suggesting the possibility of hypoglycemia. In unpublished experiments with glucose tolerance tests in carcinoid patients, it was found that although some patients exhibited a normal glucose tolerance curve, their insulin levels (radioimmuno assay) remained constant (119). It has been previously reported that carcinoid patients can exhibit impaired glucose tolerance with subnormal insulin secretion or borderline glucose tolerance with normal insulin secretion (120). The explanation for this phenomenon is that increased serotonin production, in patients with carcinoid tumor, like increased catecholamine production in patients with pheochromocytomas, may impair glucose utilization and insulin secretion. However, it is possible that other tryptophan metabolites may possess an insulin-like activity. The role of tryptophan metabolites, principally kynurenine, 3-hydroxykynurenine, hydroxyanthranilic acid, quinolinic acid, quinaldic acid and xanthurenic acid, in regulating gluconeogenesis and as diabetogenic agents had previously been suggested (53,54), while the hypoglycemic action of tryptophan metabolites via their effect on phosphoenolpyruvate carboxykinase was discussed by McDaniel in 1973 (121). The role of tryptophan metabolites still containing the intact indole portion in the hypoglycemia associated

with neoplasia was discussed by Silverstein in 1966 (52). Three patients who had hypoglycemia associated with neoplasia (fibrosarcoma, carcinoma of the esophagus and recurrent metastatic leiomyosarcoma) were studied. In these patients, increased concentrations of tryptophan and tryptophan metabolites existed in the serum and urine during hypoglycemic periods. Furthermore, intraperitoneal administration of three tryptophan metabolites, indole-3-acetic acid, indole-3-propionic acid and indole-3-butyric acid, caused profound hypoglycemia in normal and alloxan-diabetic mice. This demonstrated one of the most intriguing of all the proposed explanations for the hypoglycemia associated with neoplasia, the possibility that the tumor may elaborate a substance capable of insulin-like activity (122). To be sure, the mechanism whereby hypoglycemia occurs in neoplasia is as yet uncertain. Other explanations that have been offered include excessive metabolism of glucose by the tumor (123), excessive glycogen storage by the tumor (124,125), the presence of metastatic islet-cell tumor (126) and secretion of a beta-cell stimulating hormone (127).

The possibility, as demonstrated by Silverstein, that indolic acids may have hypoglycemic activity was particularly intriguing as carcinoid patients with large amounts of urinary indolic acids would be likely candidates for hypoglycemia associated with neoplasia. It was of interest to test some indolic acids for any hypoglycemic activity. Attention was focused on indole-3-acrylic acid because of its similarity to indole-3-propionic acid and because of its unsaturation. Bressler, in discussing the action

of hypoglycin, noted that γ,δ -unsaturated acids have hypoglycemic activity and that although acrylic acid or 2-pentenoic acid do not conform to the criterion of having an actual or potential carboxyl group separated from a carbon-carbon double bond by two carbon atoms, they are indeed hypoglycemic agents (128). Bressler suggests that these compounds are hypoglycemic by virtue of their ability to bind to and lower levels of carnitine (129) and coenzyme A which are necessary for fatty acid oxidation. The hypoglycemic effect is therefore a consequence of inhibited fatty acid oxidation and subsequent inhibition of gluconeogenesis (128). Therefore, indoleacrylic acid was tested in rats and rabbits and indolebutyric acid and indoleacetic acid in rats.

the fact that the H^+ concentration is not constant, but varies with the position x in the electrode. The concentration of H^+ is higher at the surface of the electrode ($x=0$) than in the bulk of the electrode ($x=l$). This is because the H^+ ions are consumed at the surface of the electrode, and the concentration of H^+ ions in the bulk of the electrode is higher than at the surface. The concentration of H^+ ions in the bulk of the electrode is higher than at the surface because the H^+ ions are consumed at the surface of the electrode, and the concentration of H^+ ions in the bulk of the electrode is higher than at the surface. The concentration of H^+ ions in the bulk of the electrode is higher than at the surface because the H^+ ions are consumed at the surface of the electrode, and the concentration of H^+ ions in the bulk of the electrode is higher than at the surface.

Materials and Methods

A male, New Zealand White rabbit was anesthetized with sodium pentobarbital (30mg/kg) administered intraperitoneally. Blood obtained from an ear vein was analyzed for blood sugar by a microglucose oxidase assay (Beckman). The blood sugar was monitored for 3 hr and then 86.5 mg indoleacrylic acid (50 mg/kg) (Aldrich Chemical Co.) in 2.0 ml normal saline, plus enough (ca. 0.25 ml) sodium carbonate to make the solution neutral, was administered intravenously. Blood sugar was measured for 2 hours after the injection.

Four, 200 gram male Sprague-Dawley rats were anesthetized with sodium pentobarbital (30 mg/kg) administered intraperitoneally. After measuring the control blood sugar, each rat was administered intraperitoneally one of the following compounds dissolved in 2.0 ml sodium phosphate buffer, pH 7.0: 300 mg/kg indoleacetic acid, 300 mg/kg indolebutyric acid and 300 mg/kg indoleacrylic acid. One rat was administered 2.0 ml buffer. Blood obtained from the tail vein or heart puncture was analyzed for blood sugar for 5 hr at 1 hr intervals.

Results and Discussion

Figure 57 shows the effect of indoleacrylic acid on the blood sugar of a rabbit. No lowering of the blood sugar was obtained. There was an appreciable plateauing of the blood sugar concentration for 1 hr after administration but the concentration increased after that. A problem of establishing a stable control blood sugar level in the anesthetized rabbit was noted since there was a constant increase in blood sugar from 175 mg% to 332 mg% over 3 hr. This may have been perhaps related to a failure to achieve the desired plane of anesthesia. However, it was clear that this initial experiment was inconclusive and a subsequent study was conducted more closely following the procedure of Silverstein.

Figure 58 shows the effect of indoleacrylic, indolebutyric and indoleacetic acids on the blood sugar of anesthetized rats. Figures 59 and 60 showing Silverstein's results are provided for comparison. It can be seen that I failed to duplicate Silverstein's demonstration of a hypoglycemic effect of indolebutyric acid in normal rats. If anything, I demonstrated a hyperglycemic action. The fact that the control rat maintained a blood sugar level throughout the experimental period seems to rule out any anesthesia induced hyperglycemia. It is possible that the compounds are irritative enough to raise the level of anesthesia to the point where the irritation can cause hyperglycemia. It is interesting to note that after 1 hr, the indoleacrylic acid treated rat showed a lowering of the blood sugar, although the level still had not reached the control value when the rat, for unknown reasons, died at 3 1/2 hr. There may be several

reasons for the difference between these results and those of Silverstein. The lack of number of animals involved in my experiments prevents using the results to offer any definitive explanation for these differences. Whatever the reasons behind these conflicting results, it did not seem worthwhile to test any other acids, such as 5-hydroxyindoleacrylic acid or 5-methoxyindoleacrylic acid, in this manner.

The first part of the document discusses the importance of maintaining accurate records of all transactions. It emphasizes that proper record-keeping is essential for ensuring the integrity of the financial data and for facilitating the audit process. The document outlines the various types of records that should be maintained, including invoices, receipts, and bank statements, and provides guidance on how to organize and store these records effectively.

The second part of the document focuses on the importance of regular communication and reporting. It stresses that timely and accurate reporting is crucial for providing stakeholders with the information they need to make informed decisions. The document details the reporting requirements for different types of transactions and provides examples of how to format and present the data in a clear and concise manner.

The final part of the document discusses the importance of maintaining the confidentiality and security of the financial data. It outlines the various risks associated with data breaches and provides guidance on how to implement robust security measures to protect the data. The document also discusses the importance of having a disaster recovery plan in place to ensure that the data can be recovered in the event of a system failure.

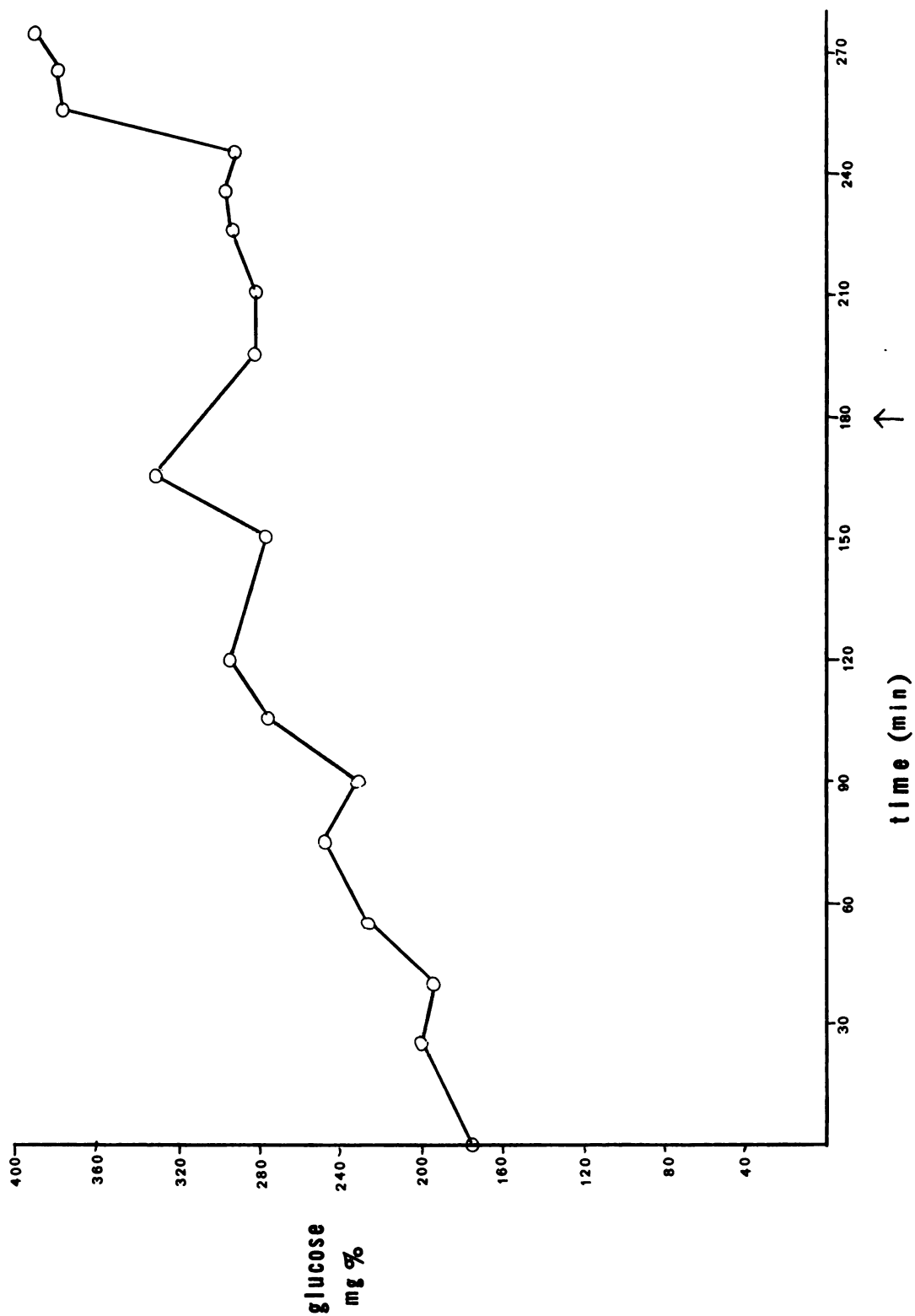


Fig. 57 Effect of Indoleacrylic acid (50 mg/kg) on blood glucose of a rabbit. (i.v. administration at 180 min)



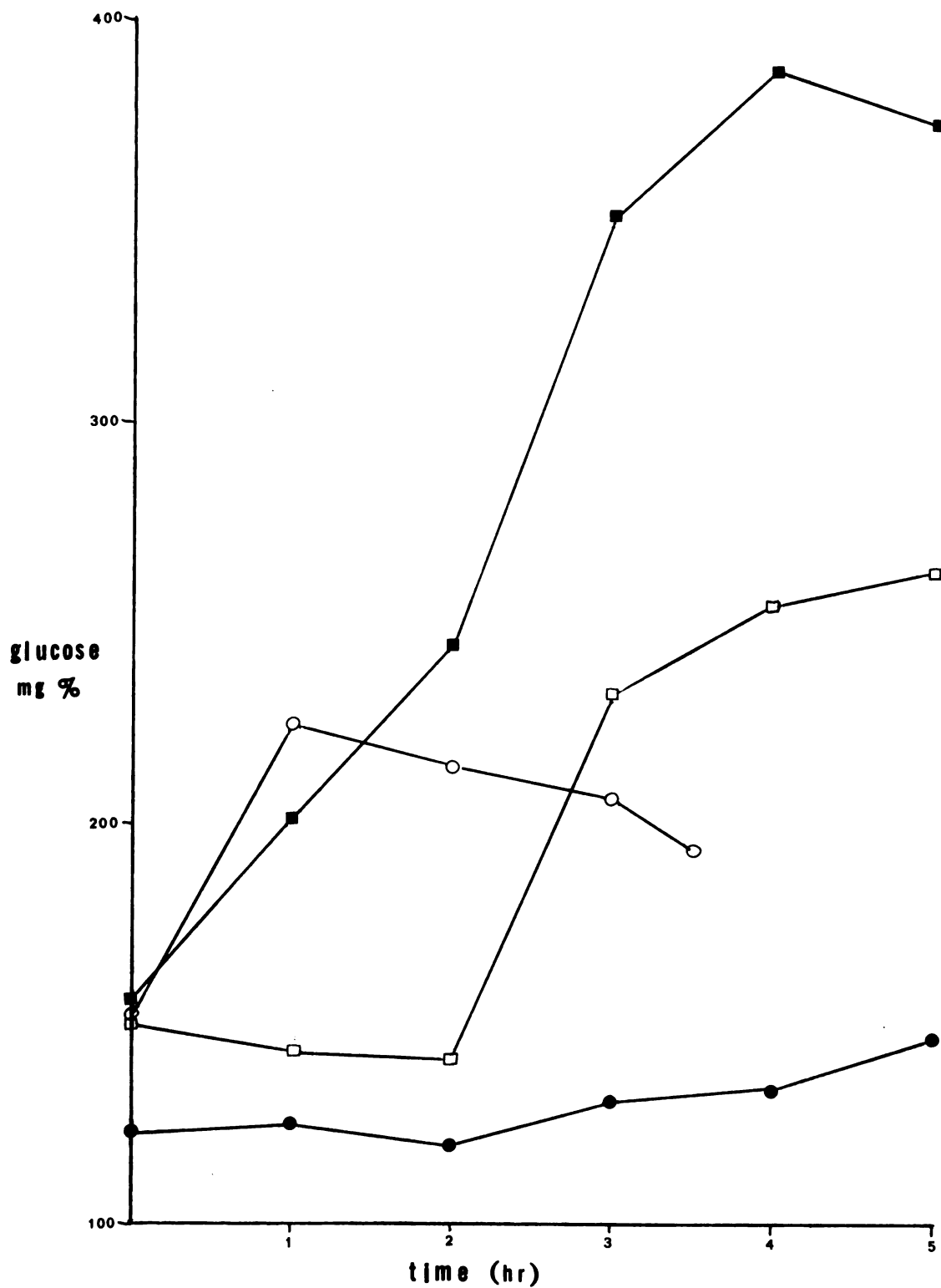


Fig. 58 Effect of indolic acids on blood glucose of rats.
 (300 mg/kg administered i.p.) □ IAA; ■ IBA;
 ○ IAcrYA; ● 0.1 M phosphate buffer.



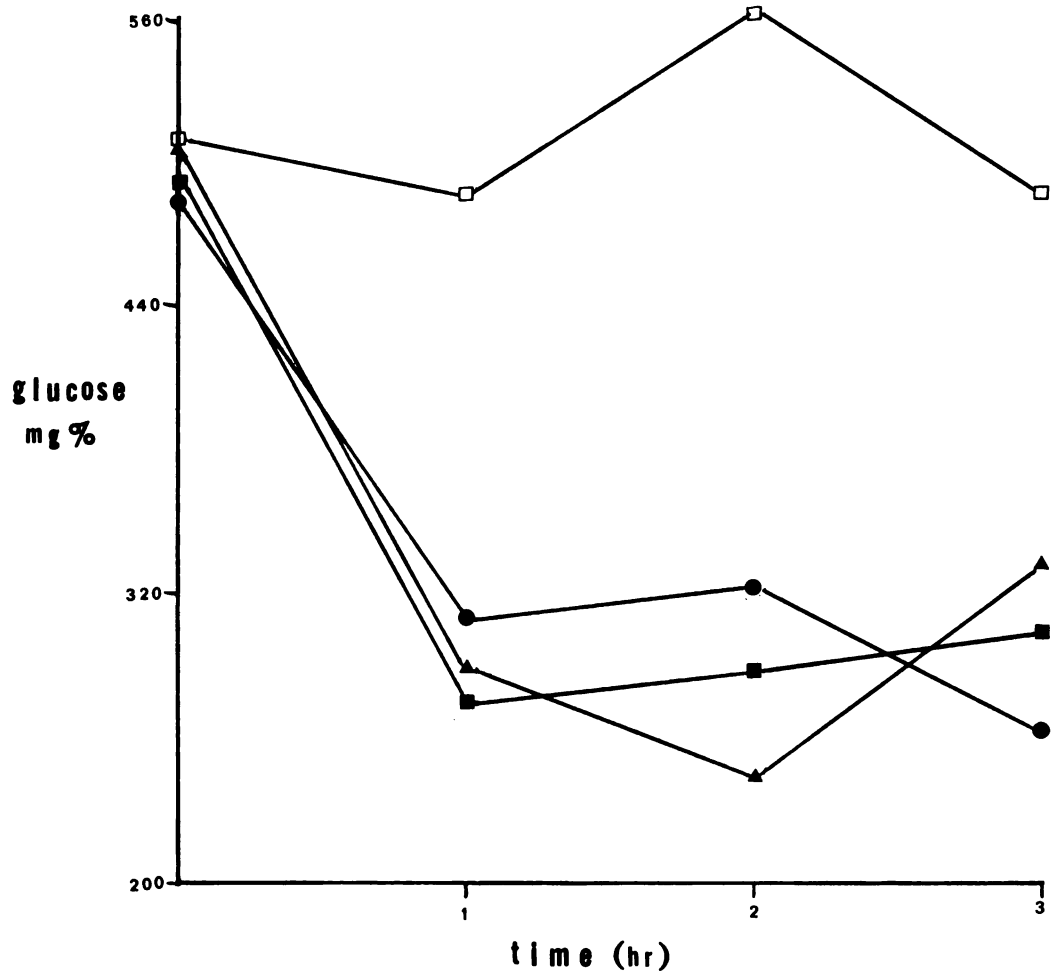
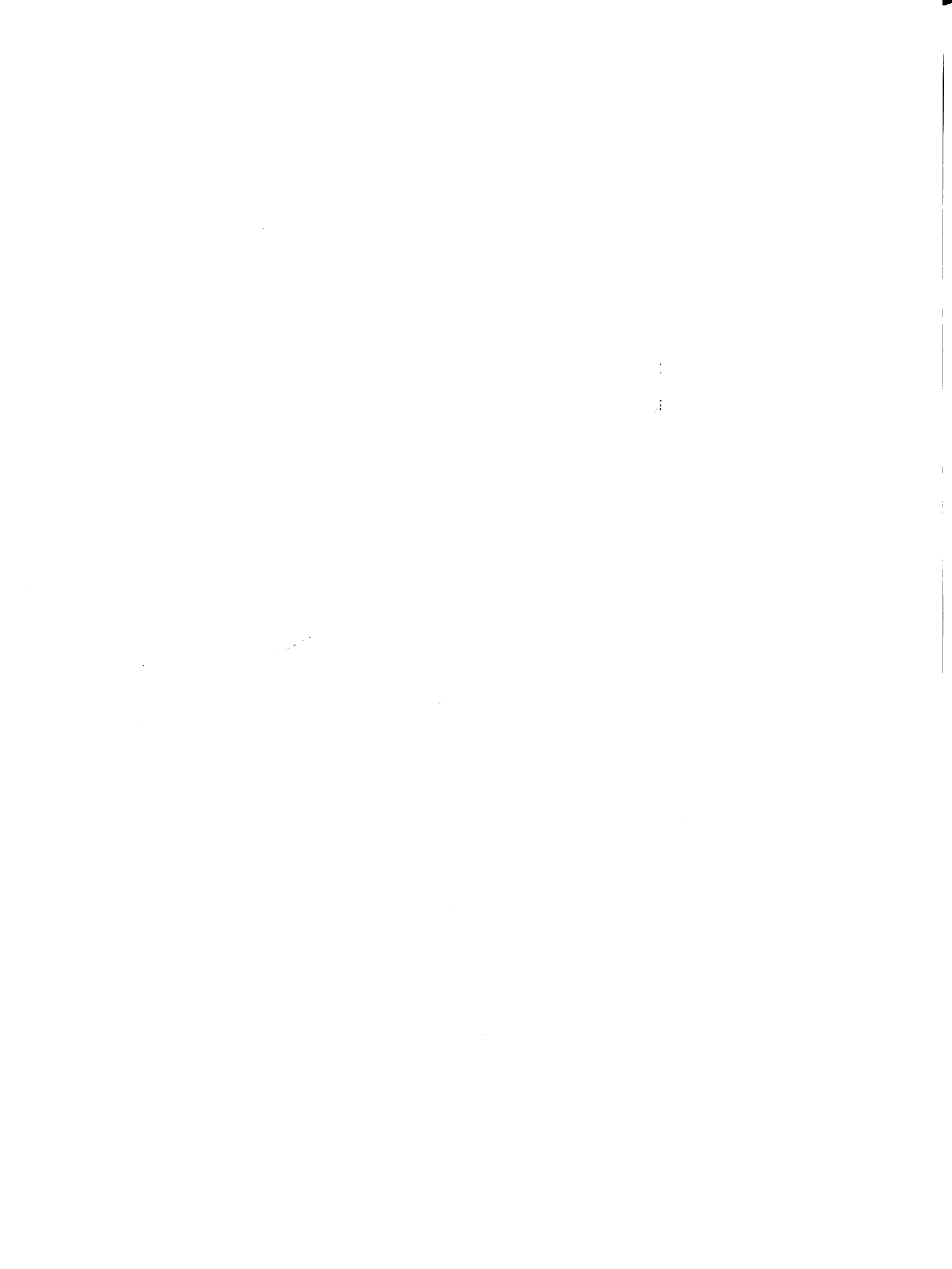


Fig. 59 Effect of indolic acids on the blood glucose of Alloxan-Diabetic mice. - (18 mice each; 400 mg/kg administered i.p.) ● IAA, ■ IPA, ▲ IBA, □ 0.5 ml 0.1 M phosphate buffer. (from Silverstein 52)



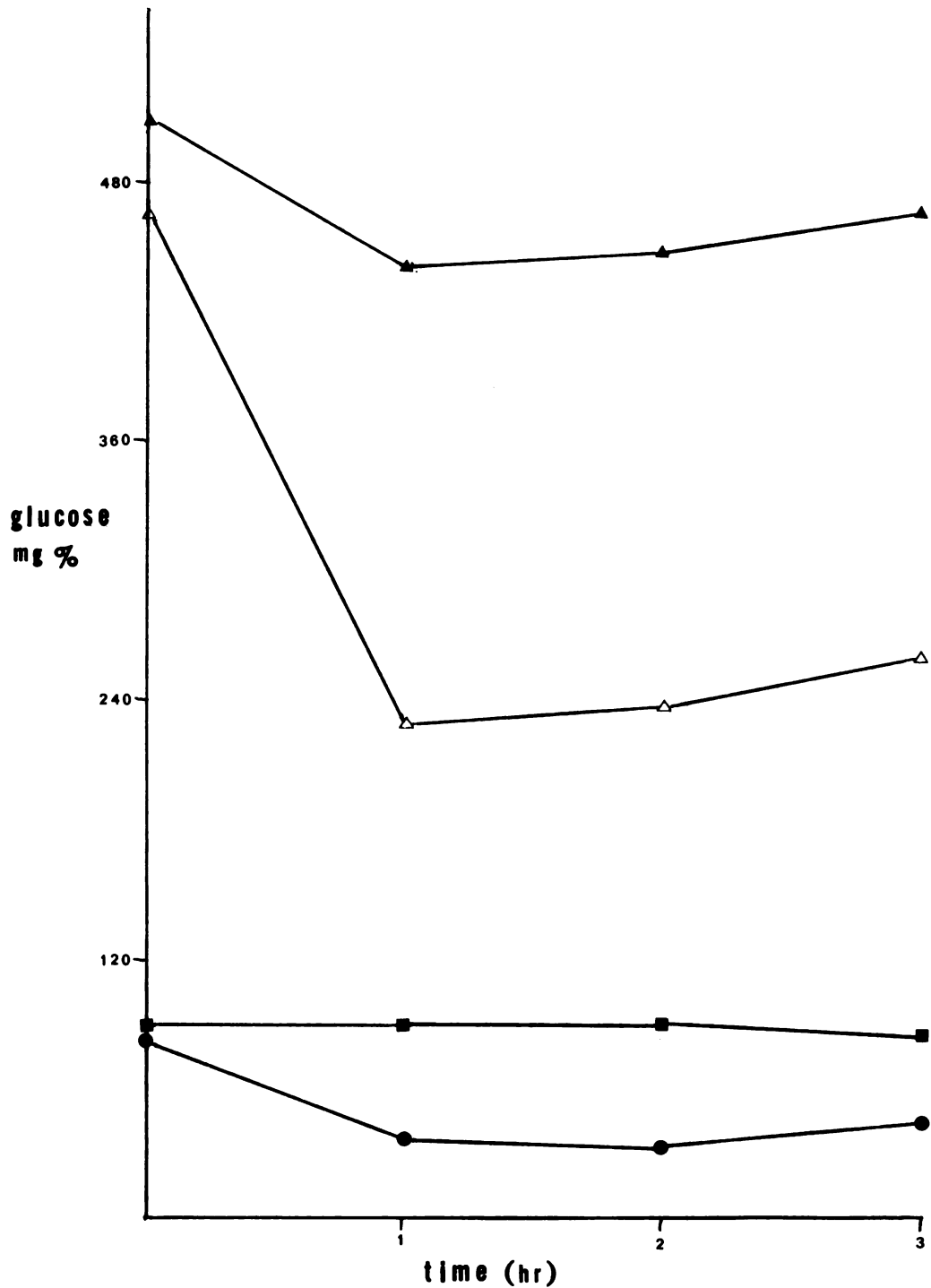


Fig. 60 Effect of IBA on blood glucose of normal and Alloxan-Diabetic rats. ● Normal rats (15), 40 mg/kg IBA; ■ Normal rats (5), 0.5 ml 0.1 M phosphate buffer; △ Alloxan-Diabetic rats (10), 40 mg/kg IBA; ▲ Alloxan-Diabetic rats (7), 0.5 ml 0.1 M phosphate buffer. (from Silverstein 52)



Part IV

E. Cell Culture

Cell Culture

An attempt was made to culture carcinoid cells in the hope that if enough cells were present, whole cell incubations with labeled tryptophan would aid in determining the in vitro metabolism of tryptophan by carcinoid tumor. A density of 10^7 cells per ml is optimum for whole cell incubations. Previously, growth of carcinoid tumors in tissue culture has been demonstrated (40).

Metastatic tumor tissue obtained using sterile techniques from the patients' liver 15 hr post mortum was sectioned as finely as possible and suspended in a T-flask in Dulbecco's modified medium with pyruvate, glutamine, fetal calves serum, penicillin, streptomycin and neomycin added. Cells were grown as a monolayer at 37°C (130).

The existance of any malignant cells growing in any culture was not confirmed by an independant pathologist.

Table 22Dulbecco's Modification (131,132)

<u>AMINO ACIDS</u>	<u>mg/litre</u>	<u>VITAMINS</u>	<u>mg/litre</u>
L-Arginine HCl	84.0	Choline Cl	4.0
L-Glutamine	580.0	Nicotinamide	4.0
L-Histidine HCl·H ₂ O	42.0	D-Ca-Pantothenate	4.0
L-Isoleucine	105.0	Pyridoxal HCl	4.0
L-Leucine	105.0	Thiamine HCl	4.0
L-Lysine HCl	146.0	Riboflavin	0.4
L-Methionine	30.0	Folic Acid	4.0
L-Phenylalanine	66.0	i-Inositol	7.2
L-Threonine	95.0	Fe(NO ₃) ₃ ·9H ₂ O	0.1
L-Tryptophan	16.0		
L-Valine	94.0		
Glycine	30.0		
L-Serine	42.0		
L-Cystine	48.0		
L-Tyrosine	72.0		

THE HISTORY OF THE

The history of the world is a vast and complex subject, encompassing the lives and actions of countless individuals and the events that have shaped our planet. From the dawn of time to the present day, the human story is one of constant change and evolution. The early civilizations of Mesopotamia, Egypt, and the Indus Valley laid the foundations of human society, with their contributions to art, science, and governance. The classical world of Greece and Rome brought forth the principles of democracy and the arts, while the Middle Ages saw the rise of the Christian Church and the development of feudalism. The Renaissance and the Enlightenment marked a period of intellectual and cultural rebirth, leading to the scientific revolution and the modern world. The 19th and 20th centuries were characterized by industrialization, global conflict, and the emergence of new nations. Today, we stand at the threshold of a new era, one defined by technological advancement and the challenges of a globalized world.

Part V

Conclusions

Conclusions

After an exhaustive screening of over twelve carcinoid patient urines by thin layer chromatography, gas-liquid chromatography and chemical ionization mass spectrometry, only one new metabolite of tryptophan was identified. One patient was found to have excreted indole-3-pyruvic acid. There was no evidence that any of the following possible metabolites of tryptophan were excreted into the urine by any of the patients that were studied: indoleacrylic acid, 5-hydroxy-indolelactic acid, 5-hydroxyindolepyruvic acid, indolepropionic acid, indolelactic acid and 5-hydroxy or 5-methoxyindoleacrylic acid.

A C.I.M.S. assay for tryptophol, 5-hydroxytryptophol and indoleacetic acid employing deuterium labeled compounds as internal standards was developed. The small number of patient urines available for assay did not enable a large enough survey to be completed so as to enable correlating of quantities of metabolites excreted with primary sites of the tumor. The assay was useful in determining tryptophol and 5-hydroxytryptophol urinary excretion in these two patients and in a Rhesus monkey.

A C.I.M.S. procedure that continuously monitors the ion current for two peaks was useful in initial screening of indolic acids present in the urine of patients but was not sufficiently accurate in its present stage of development to use as a method of quantifying the compounds under scrutiny.

1. The first part of the document discusses the importance of maintaining accurate records of all transactions.

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6. The findings of the audit should be reported to the appropriate authorities.

7. The third part of the document provides a detailed explanation of the accounting principles.

8. These principles are fundamental to the preparation of financial statements.

9. The fourth part of the document discusses the role of the auditor in the process.

10. The auditor's primary responsibility is to provide an independent opinion on the financial statements.

11. The fifth part of the document outlines the requirements for the auditor's report.

12. The report should clearly state the scope of the audit and the results of the examination.

13. The sixth part of the document discusses the ethical standards that auditors must adhere to.

14. These standards are designed to ensure the integrity and objectivity of the audit process.

15. The seventh part of the document provides a summary of the key points discussed.

16. It is hoped that this document will provide a comprehensive overview of the subject.

17. The eighth part of the document discusses the future of the profession.

18. As technology continues to advance, auditors will need to adapt to new challenges.

19. The ninth part of the document concludes with a final statement on the importance of the profession.

20. We believe that a strong and ethical auditing profession is essential for the confidence of the public.

Studies on the possible pharmacological activity of indoleacrylic acid demonstrated that this compound did not produce hypoglycemia in rats or rabbits.

Experiments using deuterium labeled tryptophan (in the 5-position) in a loading dose administered to animals demonstrated that a ratio of greater than 50:50 labeled to unlabeled must be used to prevent excessive dilution of labeled compound by endogenous tryptophan and endogenous tryptophan metabolites. The large amount of labeled tryptophan unaccounted for as metabolites raised the question of patient safety when administering large amounts of stable-isotope labeled amino acids.

In vitro studies on carcinoid tumor tissue homogenates incubated with ^{14}C -L-tryptophan were inconclusive due to the questionable viability of tumor tissue obtained 15 hr post mortum. An attempt to culture carcinoid cells failed.

An attempt to synthesize and isolate a suspected metabolite, 5-hydroxyindole-3-pyruvic acid was unsuccessful. Synthesis was probably achieved but isolation and characterization was not possible. Another suspected metabolite, 5-methoxyindole-3-acrylic acid was synthesized, isolated and characterized successfully, the first reported preparation of this compound.

Part VI

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1. The first part of the document discusses the importance of maintaining accurate records of all transactions and activities. It emphasizes that proper record-keeping is essential for transparency and accountability, particularly in financial matters. The text suggests that organizations should implement robust systems to track and document every aspect of their operations, from procurement to sales.

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3. The third part of the document focuses on the role of technology in streamlining processes and improving efficiency. It discusses various software solutions and automation tools that can help reduce manual labor and minimize errors. The text encourages organizations to embrace innovation and invest in the latest technologies to stay competitive in the market.

4. The fourth section explores the importance of collaboration and communication in achieving organizational goals. It stresses that effective teamwork and clear communication are crucial for the success of any project or initiative. The author suggests that organizations should foster a culture of open communication and encourage team members to share ideas and resources.

5. The fifth part of the document discusses the impact of external factors on organizational performance. It notes that economic conditions, market trends, and regulatory changes can all influence an organization's success. The text advises organizations to stay informed about their environment and be prepared to adapt to changing circumstances.

6. The sixth section addresses the issue of human resources and talent management. It emphasizes the need for organizations to attract, develop, and retain top talent. The author suggests that organizations should invest in training and development programs to ensure that their workforce is equipped with the skills and knowledge needed to succeed in a competitive market.

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10. The tenth and final section of the document discusses the importance of sustainability and social responsibility. It notes that organizations have a responsibility to their stakeholders and the environment. The author suggests that organizations should adopt sustainable practices and contribute to the well-being of the community.

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6. The third part of the document provides a detailed overview of the financial statements and their components.

7. This section includes a breakdown of the income statement, balance sheet, and cash flow statement.

8. The fourth part of the document discusses the impact of external factors on the organization's performance.

9. It highlights the need for strategic planning and risk management to navigate these challenges effectively.

10. The fifth part of the document concludes with a summary of the key findings and recommendations.

11. It emphasizes the importance of continuous monitoring and evaluation to ensure long-term success.

12. The final part of the document provides a list of references and sources used in the analysis.

13. This section includes a bibliography of relevant literature and industry reports.

14. The document is intended to serve as a comprehensive guide for stakeholders and decision-makers.

15. It is hoped that the information provided will be valuable in informing future actions and strategies.

16. The author expresses gratitude to the individuals and organizations that provided support and data during the research process.

17. The document is subject to change based on new information and developments in the field.

18. It is recommended that the findings be reviewed and updated regularly to reflect current trends and insights.

19. The author reserves the right to use the information contained herein for future publications and reports.

20. The document is a confidential document and its contents should not be disclosed to unauthorized parties.

21. It is the property of the organization and should be handled accordingly.

22. The author acknowledges the limitations of the study and the need for further research in certain areas.

23. The document is a work in progress and may be subject to further revisions and updates.

24. It is intended to provide a snapshot of the current state of affairs and to guide future decision-making.

25. The author is available for consultation and further information regarding the findings and recommendations.

26. The document is a valuable resource for anyone interested in the subject matter and its implications.

27. It is hoped that the information provided will be helpful in achieving the organization's goals and objectives.

28. The author expresses a commitment to transparency and accountability in all aspects of the work.

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7. This section includes a breakdown of the income statement, balance sheet, and cash flow statement.

8. The fourth part of the document discusses the various methods used to calculate and analyze financial ratios.

9. These ratios are used to assess the company's financial health and performance over time.

10. The fifth part of the document covers the process of budgeting and forecasting future financial outcomes.

11. It emphasizes the need for realistic assumptions and regular monitoring of the budget against actual results.

12. The sixth part of the document addresses the importance of risk management and identifying potential threats to the organization.

13. This section discusses various risk mitigation strategies and the role of insurance in protecting the company's assets.

14. The seventh part of the document provides a summary of the key findings and conclusions from the analysis.

15. It highlights the areas where the company is performing well and identifies opportunities for improvement.

16. The eighth part of the document offers recommendations for future actions and strategies to enhance the company's financial performance.

17. These recommendations are based on the findings of the analysis and are tailored to the specific needs of the organization.

18. The ninth part of the document provides a final overview of the document's structure and content.

19. It serves as a reference for readers who may want to explore specific sections in more detail.

20. Finally, the document concludes with a statement of appreciation for the support and cooperation of all stakeholders.

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7. The third part of the document provides a detailed analysis of the data collected.

8. This analysis reveals several trends and patterns in the data.

9. The results indicate a significant increase in activity over the period.

10. The fourth part of the document discusses the implications of these findings.

11. It is concluded that the data supports the initial hypothesis.

12. Further research is recommended to explore these findings in greater depth.

13. The fifth part of the document provides a summary of the key points.

14. The overall conclusion is that the data is highly reliable and consistent.

15. The sixth part of the document discusses the limitations of the study.

16. It is noted that the sample size was relatively small.

17. The seventh part of the document provides a list of references.

18. The references include several key works in the field.

19. The eighth part of the document provides a list of appendices.

20. The appendices contain additional data and supporting information.

21. The ninth part of the document provides a list of footnotes.

22. The footnotes provide further details on the methodology used.

23. The tenth part of the document provides a list of acknowledgments.

24. The acknowledgments thank the individuals who assisted in the study.

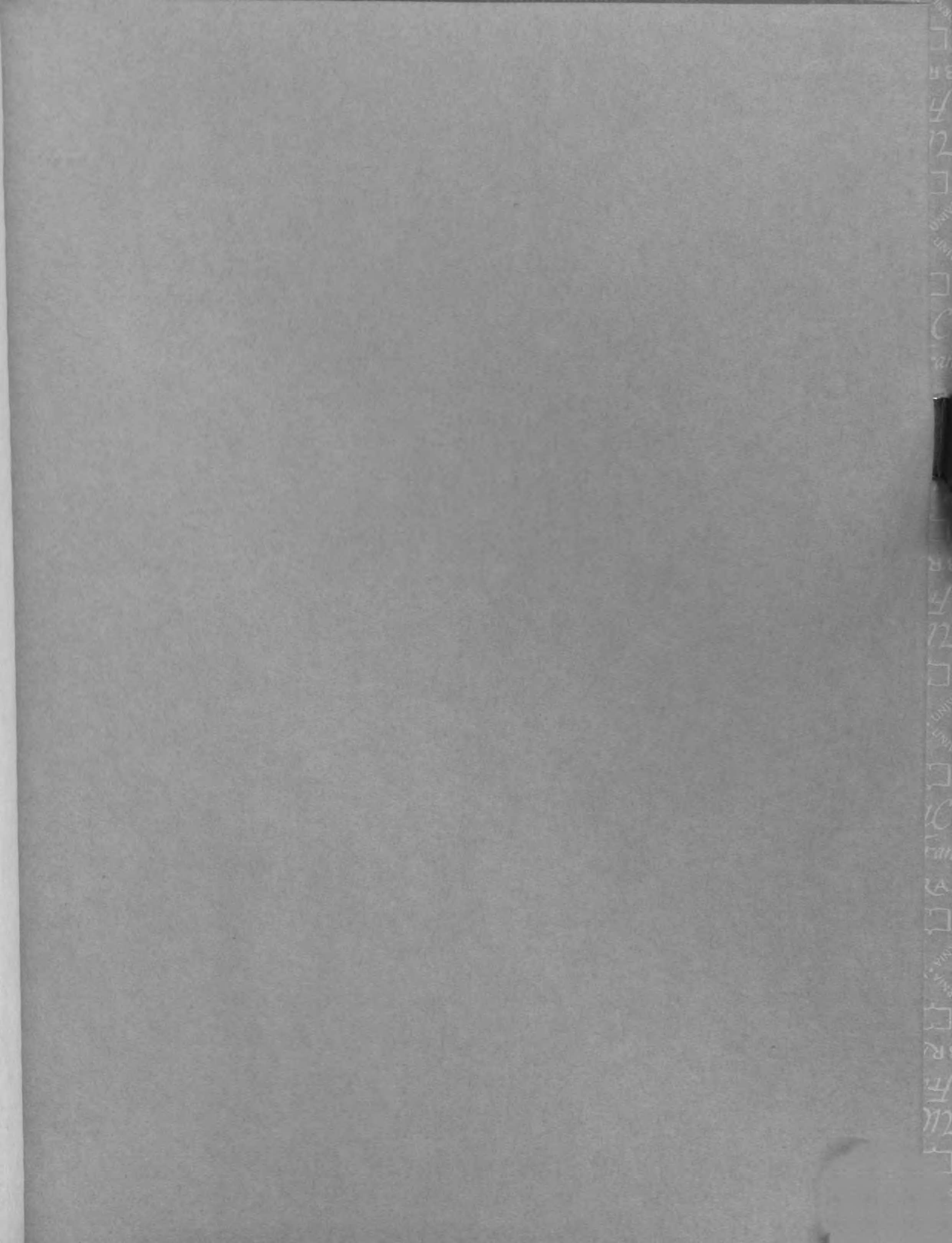
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26. The contact information includes the author's name and affiliation.

27. The twelfth part of the document provides a list of keywords.

28. The keywords are used to describe the main topics of the document.

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