THE MECHANISM OF ACTION AND EFFECTS OF THE NIACIN ANALOGUE 6-AMINONICOTINIC ACID IN *ESCHERICHIA COLI*

By

JOHN RONALD COBB

Bachelor of Science

Oklahoma State University

Stillwater, Oklahoma

1972

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
July, 1974

THE MECHANISM OF ACTION AND EFFECTS OF THE NIACIN ANALOGUE 6-AMINONICOTINIC ACID IN *ESCHERICHIA COLI*

Thesis Approved:

Robert K. Gholson
Thesis Adviser

Roger E. Koepke

Franklin R. Leach

D. D. Drake
Dean of the Graduate College

ACKNOWLEDGEMENTS

The author extends thanks and appreciation to his major professor, Dr. R. K. Gholson, for his meaningful and stimulating advise during this work and preparation of this thesis. Appreciation is also extended to Dr. F. R. Leach and Dr. R. E. Koeppe for their contributions as members of my committee. Thanks is also extended to Dr. Prem Juneja and Dr. Jerry Chandler for their help in the preparation and isolation aspects of this work.

The author extends his gratitude to Miss Susan Pearcy and Miss Donna Wilson for their valuable assistance during the course of these experiments; to Ms. Lisa Thompson for her effort in typing this manuscript; to Gretchen Henderson for her work in preparation of the figures.

The financial support and provision of facilities by the Department of Biochemistry and by the author's parents are gratefully acknowledged.

A very special note of thanks and appreciation is extended to my wife Beverly and my daughter Amy, for their love which has made this study worth completing.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

LIST OF ABBREVIATIONS

6ANA	6-Aminonicotinic acid
6ANam	6-Aminonicotinamide
6ANAD	6-Aminonicotinamide adenine dinucleotide
6ANMN	6-Aminonicotinamide mononucleotide
ARPPR	Adenosine diphosphate ribose
des 6ANAD	6-Aminonicotinic acid adenine dinucleotide
des NAD	Nicotinic acid dinucleotide
DHAD	Dihydroxyaceton phosphate
NA	Nicotinic acid
NAD	Nicotinamide adenine dinucleotide
NADase	Nicotinamide adenine dinucleotide glycohydrolase
NADP	Nicotinamide adenine dinucleotide phosphate
Nam	Nicotinamide
NaMN	Nicotinic Acid mononucleotide
NMN	Nicotinamide mononucleotide
PRPP	5-Phosphoribosyl-1-pyrophosphate
QA	Quinolinic acid
PABA	p-Aminobenzoic acid

CHAPTER I

INTRODUCTION

6-Aminonicotinic acid (6ANA) inhibits the growth of Escherichia coli. One possible mechanism is metabolism of 6ANA into the analogue of NAD, 6-aminonicotinamide adenine dinucleotide (6ANAD). This analogue in turn could stop the de novo biosynthesis of NAD by repression and feed back inhibition. Also 6ANAD inhibits some NAD dependent oxido-reduction enzyme reactions. Unlike many NAD analogues 6ANAD is not reducible.

The effects of 6ANA and 6ANam have been studied extensively in mammals. The mechanism of formation of 6ANAD in mammals is through the NAD glycohydrolase exchange reaction; however, this enzyme has not been detected in E. coli. Therefore, the 6ANA or 6ANam must be metabolized by the pyridine nucleotide cycle for conversion into 6ANAD.

In this study an E. coli mutant W3899n (nad B and pnc A) which cannot produce NAD by the de novo pathway has been used. It also cannot deaminate Nam to NA. Therefore, the mutant must have an exogenous source of NA for growth.

The following studies were carried out to determine the bacteriostatic mechanism of this compound (6ANA). 6ANA-^{14}C was synthesized and incubated with W3899n. The medium and perchloric acid soluable cell fraction were then analyzed using conventional chromatography techniques.

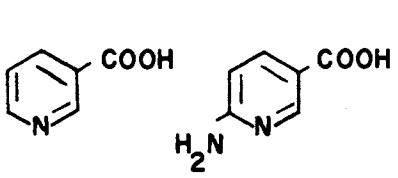
CHAPTER II

LITERATURE REVIEW

NAD (nicotinamide adenine dinucleotide) and NADP (nicotinamide adenine dinucleotide phosphate) are cofactors to biological oxido-reduction reactions (1). These cofactors function by accepting an hydronium ion at the 4 position of the pyridine ring, (2) and donating that hydronium ion to another compound in a reduction reaction. These cofactors, NAD and NADP, and their reduced forms, NADH and NADPH, participate in over 200 different biological oxido-reduction reactions (1).

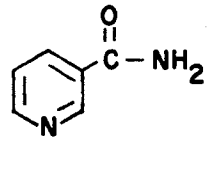
NAD was initially isolated as a dialyzable cofactor required for alcohol production from glucose in yeast extracts by Harden and Young in 1904 (2). This cofactor ("cozymase" or "coenzyme I") contains nicotinamide as established by Albus, Schlenk, and von Euler in 1935 (3). Another dialyzable cofactor ("coferment" or "coenzyme II") which also contained nicotinamide was found by Warburg and Christian (4,5) in 1934. These cofactors were different because coferment could not substitute for cozymase in the production of alcohol from glucose. The elucidation of the structure of these cofactors, NAD and NADP, was accomplished by von Euler *et al.*, (6,7) and Schlenk (8) in 1937. The hydrogen transfer abilities of NAD were elucidated by Warburg and Christian (5,9,10), with Pullman *et al.* later showing that the reduction takes place at the 4 position (11).

Figure 1. Structures of Nicotinic Acid, 6-Aminonicotinic Acid, Nicotinamide, 6-Aminonicotinamide, Pyridine Nucleotides, and Pyridine Nucleotide Analogue

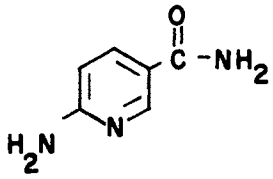


NA

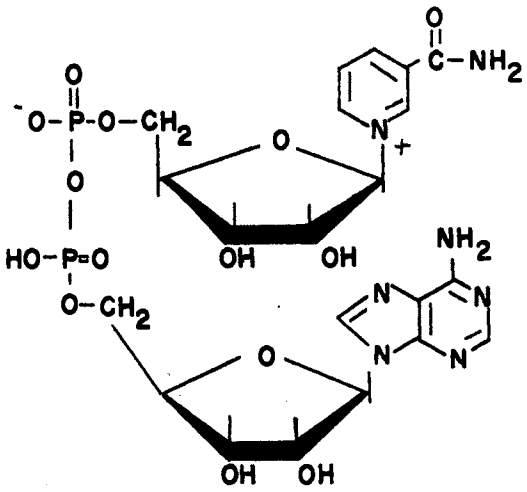
6ANA



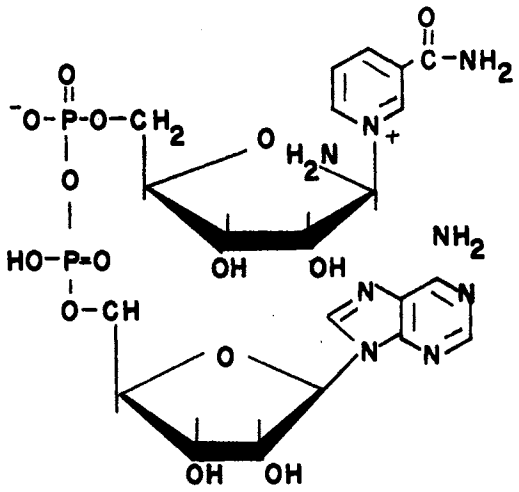
Nam



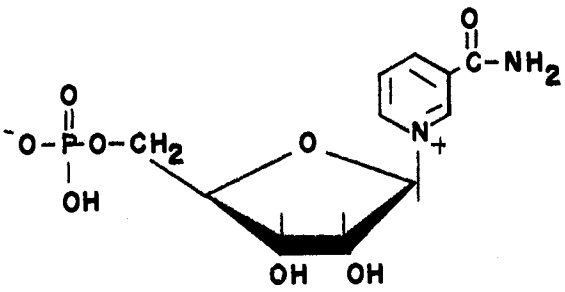
6ANam



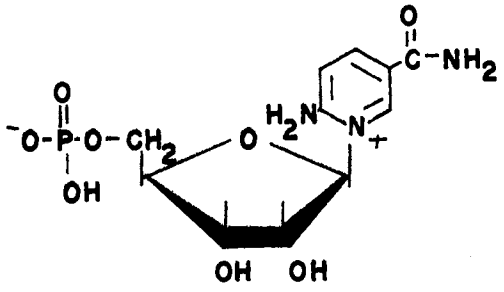
NAD



6ANAD



NMN



6ANMN

Biosynthesis of NAD

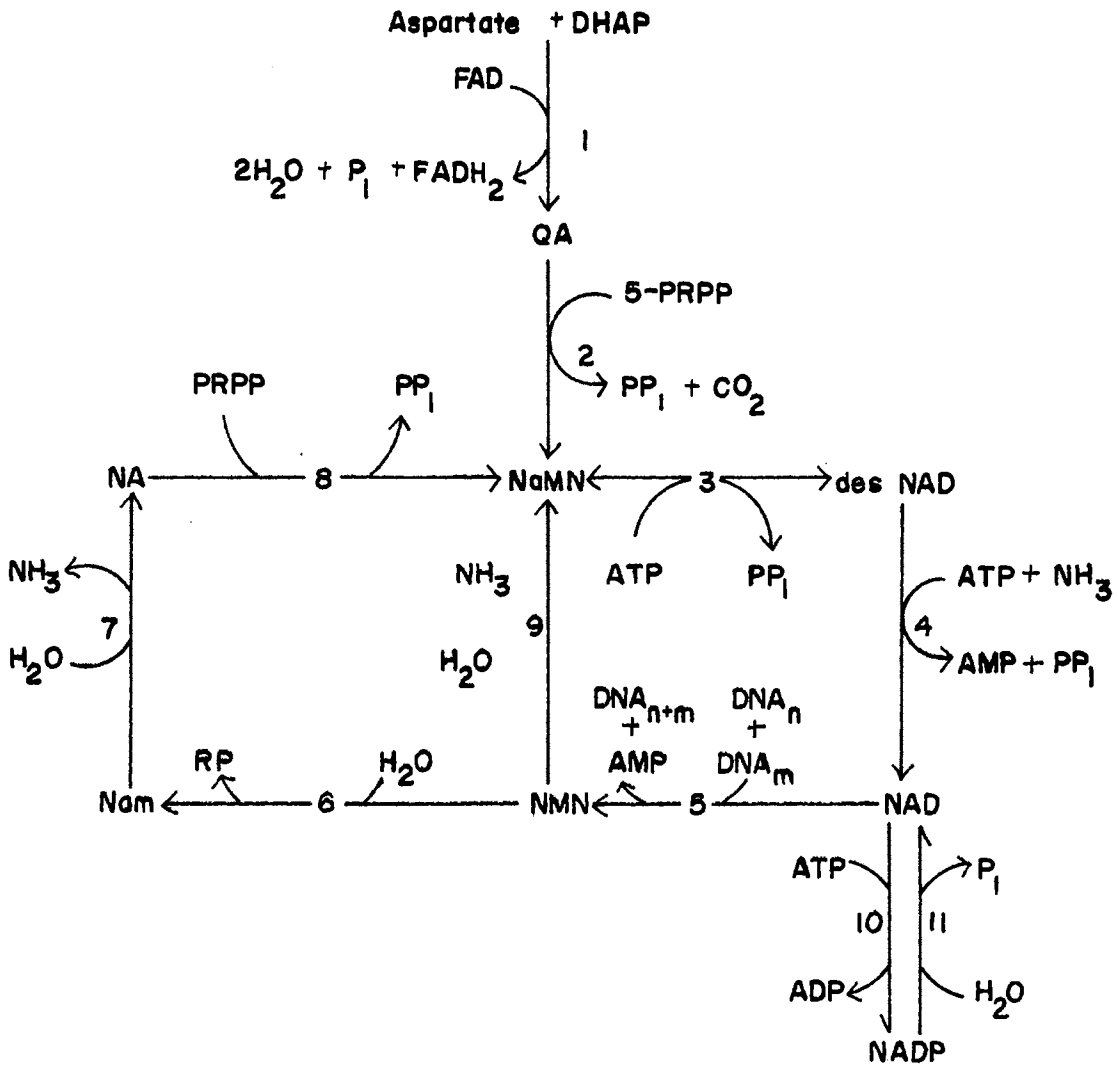
While the structure and many of the properties of NAD were elucidated by 1954, major features of the de novo biosynthesis were not elucidated until the 1960's. The de novo biosynthesis begins with the condensation of aspartate and dihydroxyacetone-phosphate to form quinolinic acid (QA) (12, 13, 14). QA is converted to NaMN with the addition of PRPP and loss of CO_2 (15). NaMN in turn is converted with the addition of AMP from ATP to desamido NAD which is then amidated to form NAD (16). NADP is produced by the reaction NAD + ATP NADP + ADP (17). These reactions are summarized in Figure 2.

Pyridine Nucleotide Cycle

E. coli also contain nicotinamide deamidase and NaMN pyrophosphorylase, allowing conversion of Nam to NA and NA to NaMN (18). A general pyridine nucleotide cycle was proposed by Gholson in 1966 (19). This cycle, NaMN des NAD NAD Nam NA NaMN, allows the recovery of Nam formed in the breakdown of NAD.

The degradation of NAD occurs during repair, recombination, or synthesis of DNA. A DNA ligase joins strands of DNA at the expense of one NAD per junction in the reaction $NDA_n + DNA_m + NAD \quad DNA_{n+m} + NMN + AMP$ (20). There has been no evidence of any other enzymes in E. coli that cleave NAD into NMN and AMP or Nam and ADP-ribose (21). The latter reaction is catalyzed by NAD glycohydrolase which is present in Mycobacterium butyricuin (22) and Bacillus subtilis (23). Any NMN produced from the ligase reaction is either converted into NaMN or into Nam and ribose-5-phosphate (21). NMN amidohydrolase deamidates NMN to

Figure 2. The Pyridine Nucleotide Cycle in E. coli



1. Quinolinate Synthetase (A & B protein)
2. Quinolinate Phosphoribosyl Transferase
3. des NAD Pyrophosphorylase
4. NAD Synthetase
5. DNA Ligase
6. NMN Glycohydrolase
7. Nicotinamide Deamidase
8. NaMN Pyrophosphorylase
9. NMN Amidohydrolase
10. NAD Kinase
11. NADP Phosphotase

NaMN producing a cycle within a cycle. Olivera has argued that this cycle: NaMN des NAD NAD NMN NaMN is the major pathway of pyridine nucleotide turnover (24). NMN glycohydrolase splits NMN into Nam and R-5-P also completing a pyridine nucleotide cycle: NaMN des NAD NAD NMN Nam NA NaMN.

Genetic Studies of de novo Pathway and Pyridine Nucleotide Cycle

The de novo pathway and the pyridine nucleotide cycle have been studied using several E. coli mutants. Threee genes in the de novo pathway have been defined: nad A, nad B, and nad C. The nad A gene maps near minute 17 on the E. coli chromosome and produces quinolinate synthetase A protein (25). That is, QA synthetase B activity is present but A activity is not; also QA phosphoribosyl transferase is present in nad A mutants. The nad B genes map between pur I and tyr A at minute 48 (25, 26). These mutants can be divided into two groups, these that complement nad A in vitro and those which do not. The latter lack both QA synthetase A and B protein activities and are presumable regulatory mutants. This class of mutants has been tentatively designated as nad R.

The nad C mutants lack QA phosphoribosyl transferase. This gene is located between leu and pan at approximately minute 1.5 on the genetic map (27). Nad C mutants excrete large amounts of QA into the medium but addition of pyridine derivatives such as NA, Nam, and NAD, in concentrations greater than 2×10^{-6} molar inhibits this excretion. The inhibition is due to repression of both the nad A and nad B structural gene products (28).

Tentative evidence suggests that NAD is the metabolically active feedback inhibitor of QA synthetase.

A mutant blocked within the pyridine nucleotide cycle-pnc A-at the nicotinamide demidase step has been isolated (29). This mutant was produced from a strain (w3899) of E. coli which contained a nad B mutation, producing a double mutant (w3899n) blocked in the de novo pathway and requiring NA for growth but not able to utilize Nam for growth.

Analogs of NAD

Analogs of NAD have been used to elaborate the structure of reduced NAD, the structure of NADP, in studies on dehydrogenases, and in studies on carcinostatic NAD analogs. N-methylnicotinamide and other quaternary pyridinium coupounds were used to elaborate the structure of reduced NAD in 1937 (30, 31). The position of the 2' monoester phosphate group on NADP was determined by using the 3' isomer of NADP which was inactive with enqymes which require NADP but not with one which uses either NAD or NADP (32). Studies on dehydrogenases have also utilized deoxyadenylic NAD (33) and dinicotinamide Ribose 5' -pyrophosphate (34).

Several analogs of the Nam portion of NAD have also been produced using animal tissue NAD glycohydrolase (NADase). Only animal NADase will catalize the exchange reaction $ARPPN^{+} + X \quad ARPPX^{+} + N$ (35). This reaction is inhibited 50% by 1.5×10^{-3} M nicotinamide. Neurospora also contains a NAD glycohydrolase which catalyzes NAD hydrolysis but not the exchange reaction and is not affected by nicotinamide (36). The isonicotinic acid hydrazide analog of NAD was the first such

compound studied (36). This analog is not enzymatically reducible but 10^{-3} M analogue has no significant effect on the rate of reduction of NAD by yeast alcohol dehydrogenase, horse liver alcohol dehydrogenase, transhydrogenase of Pseudomonas, or rabbit muscle lactate dehydrogenase (37). This analogue can be cleaved by NADase.

The 3-acetylpyridine analogue of NAD has also been produced using the same reaction as with INH (38). This analogue, howver, is enzymatically reducible, as the 4 position is available for reduction. The rate of reduction is only 1/20 that of NAD with yeast alcohol dehydrogenase. Other analogs are lested in Table I.

6ANA and 6ANam in Mammallian Systems

Much work has been done with 6ANA and 6ANam in mammals since it was found toxic to rabbits and rats in 1955 (40). At low dosage levels 6ANam caused paralysis of the extremities and blindness. Further studies with rats confirmed the delayed effect of the compound, and it was concluded that 6ANam was an antimetabolite of Nam (41). Shapiro, Bietrich, and Shils tested 6ANam for carcinostatic activity against the 755 mammary adenocarcinoma of mice because 6ANam was such a potent antagonist of Nam (42). 6ANam exerted strong antitumor activity against the mammary adenocarcinoma and this effect was completely reversed by the administration of nicotinamide. Further, radiotherapy in combination with treatment with 6ANam was effective in damaging the tumors (43).

The mechanism of action of 6ANam was postulated as conversion to the nonphysiological nucleotide analogues of NAD and NADP. This analogue formation may be irreversible, thus, not allowing the tissue

TABLE I[a]

COMPARISON OF REACTIONS OF NAD WITH SOME 3-SUBSTITUTED PYRIDINE ANALOGUES OF NAD

-X N-R	Reaction with cyanide	Reduced by yeast alcohol dehydrogenase	Reduced by hydrosulfite
X			
$-\overset{O}{\overset{\|}{C}}-NH_2$	+	+	+
$-\overset{O}{\overset{\|}{O}}-CH_3$	+	+	+
$-\overset{O}{\overset{\|}{C}}-H$	+	+	+
$-\overset{O}{\overset{\|}{C}}-O-C_2H_5$	+	-	+
$-\overset{O}{\overset{\|}{C}}-\overset{C_2H_5}{\overset{\|}{N}}-H$	+	-	+
$-\overset{OH}{\overset{\|}{C}}-CH_3$ H	-	-	-
$-CH_3$	-	-	-

[a] (39)

to rid itself of the analogue (37). 6ANam was converted to 6ANAD and 6ANADP *in vivo* and *in vitro* and the effects of these analogues were tested on several NAD dependent enzymatic systems in the 755 tumor. The activities of 3-phosphoglyceraldehyde dehydrogenase, the conversion of B-hydroxybutyrate to acetoacetate, and -ketoglutarate oxidase were markedly inhibited, while lactic acid dehydrogenase was unaffected (44). 6ANam lowers the level of ADP and ATP and greatly increases the concentration of AMP in the 755 tumor.

A study (Table II) on the energies of molecular orbitals of NAD, NADH, and 6ANAD revealed that the lowest empty electronic orbital is placed much higher in 6ANAD than in NAD (45). Thus, the analogue does not possess the pronounced electron-acceptor properties of the true coenzyme and cannot function as an electron carrier in the respiratory chain or in other oxidation-reduction reactions (45).

6ANam has also been used to study the hexose-monophosphate shunt as related to glucose induced insulin release, because 6ANam markedly reduces oxidation of (1-^{14}C) glucose but does not affect oxidation of glucose labelled in C-6. Also pretreatment with 6ANam does not change the insulin concentration in the test animals (46, 47).

Further studies in 6ANam in animal systems include studies on behavioral changes in rats (48), effects on erythrocytes (49), effects on Na^+ and K^+ active transport in rat tubular cells (50), and effects of 6ANam in the presence of rat brain microsome nucleosidases (51).

In mammals, the incorporation of 6ANam into 6ANAD does not occur primarily through the pyridine nucleotide cycle, but through exchange with the NAm portion of NAD by NAD glycohydrolase (51). To reiterate, this enzyme has not been found in *E. coli*. One result of this exchange

TABLE II[a]

ENERGIES OF MOLECULAR ORBITALS

	Compounds		
	NAD^+	NADH	$6ANAD^+$
Energy of Lowest Empty Molecular Orbital (ev)	-0.356	-0.915	-0.471
Energy of Highest Occupied Molecular Orbital (ev)	1.032	0.298	0.735

[a] (45)

reaction is that in mammals the concentration of 6ANA must be seven times that of 6ANam to produce the same effects (52).

6ANA and 6ANam in Bacterial Systems

Limited work has been done with 6ANA in bacterial systems. 6ANA was first studied as an analogue of p-aminobenzoic acid. It was bacteriostatic to E. coli and Streptococcus hemolyticus but not to Diplococcus pneumoniae (53). These effects were reversed by PABA.

In 1970 the folic acid analogue containing 6ANA was synthesized to study its antineoplastic activity (54). The analogue was inactive against leukemia L1210 in mice and noncytotoxic when tested against HE_p-2 cells in culture. The analogue supported growth of Streptococcus faecalis.

In a study on bacteriophage in 1951 6ANA suppressed the multiplication of the phage but the inhibition could be reversed by Nam or PABA or acetic acid (55).

CHAPTER III

METHODS AND MATERIALS

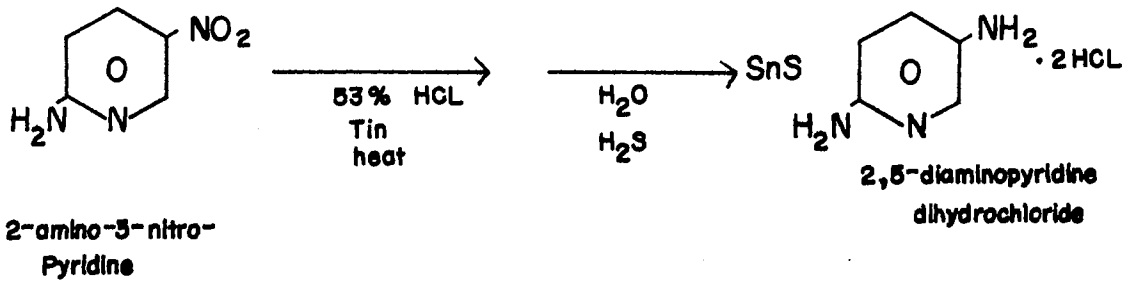
Synthesis of 6ANA-^{14}C and 6ANam-^{14}C

The procedure (Figure 3) of Wacker, Lochmann, and Trager (56) was used in the preparation of 6ANA-^{14}C. This procedure was also used to prepare 6ANam-^{14}C except that the 2-amino-5-cyano-pyridine-^{14}C was not hydrolyzed with NaOH. Instead, Dowex 50 hydrogen form was added to the reaction mixture and heated at 100^{o} for one hour. 6ANam-^{14}C was removed from the resin with water. The specific activity of the 6ANA-^{14}C was 3.2 millicuries per millimole and the specific activity of the 6ANam-^{14}C was 2.7 millicuries per millimole. The purity of each was checked by autoradiography using thin layer chromatography systems I and II (57).

Cultures

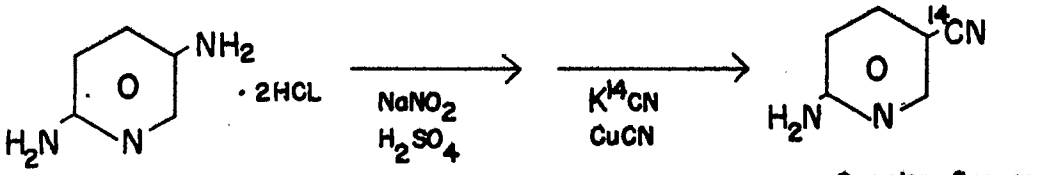
The E. coli mutants, W3899 and W3899n, were maintained in nutrient agar slab cultures. New nutrient agar plate cultures used for inoculation were prepared monthly. Each mutant was checked for its genetic characteristics before each experiment. Experimental cultures were grown in the minimal medium of Yates and Pardee (58) with vigorous shaking at 37^{o}. The only addition to this medium was 10^{-6} molar NA. The bacteria were gorwn in volumes of 25 ml, 100 ml, 500, and 10 liters.

Figure 3. Synthesis of 6ANA-^{14}C and 6ANam-^{14}C
See Text for Experimental Details.



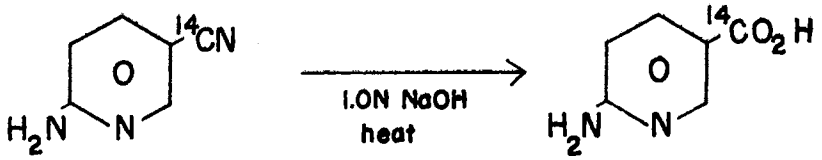
2-amino-5-nitro-
Pyridine

2,5-diaminopyridine
dihydrochloride



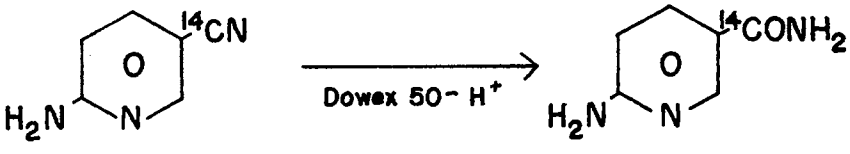
2,5-diaminopyridine
dihydrochloride

2-amino-5-cyano
pyridine



2-amino-5-cyano pyridine

6-aminonicotinic acid



Each culture was inoculated from a starter culture of 5% its volume. When the concentration of cells reached approximately 10^9 cells/ml the suspension was centrifuged on a Sorvall RC2-B at 15,000 rpm for 15 minutes at 4^o in a SS-34 head. The 10 liter batch was harvested with a Sharples centrifuge. The bacteria were washed with 0.9% NaCl.

Test Cultures

The washed cells were resuspended in minimal medium in amounts equal to those from which they were harvested. 2 X 10^{-4}M 6ANA-^{14}C and 10^{-4}M PABA or an equal concentration of control compound, NA-^{14}C, Nam-^{14}C, or 6ANam-^{14}C were added to the media. The cultures were shaken at 37^o for six hours, harvested, washed and suspended in deionized water at five milliliters per gram. The suspended cells were sonicated at 4^o with a Branson Sonifier at 10 amperes DC for one minute per 20 milliliters. An equal volume of 15% perchloric acid was immediately added to the sonicate. The solution was centrifuged in a Sorvall centrafuge for 30 minutes at 20,000 rpm in a SS-34 head. The supernatant was neutralized to pH 7 with 10N KOH and centrifuged at 4^o. This supernatant was analyzed by ion exchange chromatography.

Ion Exchange Chromatography

The medium in which the cells were incubated for six hours and the cell extracts were passed through Dowex 1 X 8 formate columns (3cm X 60 cm). Water and varying normalities of formic acid were used to elute the various compounds. In the case of the medium, cold authentic carrier (6ANA and 6ANam - final concentration 10^{-2}M) were added except when noted. The fractions (10mls) from the columns were

collected on a Buchler fraction collector and absorbance, conductivity on a Radiometer conductivity meter, and radioactivity were measured.

Some fractions were further purified on a Bio-Rad cellex-D anion exchange cellulose column (1cm x 40cm) using a NH_4HCO_3 gradient from 0 M to 0.4 M for elution.

Spectroscopic Methods

Spectroscopic studies were made with a Beckman DB spectrophotometer. UV and visible light scans of 6ANA and NA compounds were run using a 0.1 M phosphate buffer at the noted pH's. Columns fractions were monitored at A_{260} unless otherwise specified, using a Gilson automatic transferator and a water blank.

Liquid Scintillation

Radioactivity was determined using liquidscintillation counting. Samples were added to 10 ml of counting liquid in scintillation vials and assayed on a Packard Tri-Carb liquid scintillation spectrometer series 314A or model 3003. The scintillation liquid was a mixture of 4 grams PPO, 0.2 grams POPOP, 400 ml ethanol, and 600 ml toluene making one liter. The dpm of each sample was calculates using the channels ratio method for quench correction and counting efficiency. Quantatative calculations were made assuming the specific activity of the labeled remained constant.

Thin Layer Chromatography

Thin layer chromatography involved the use of Analtech, Inc. cellulose glass plates, 250 microns thick with fluorescent indicator.

Also, Brinkmann Polygram cel 300 PEI plates were used. Whatman DE81 paper was used for all paper chromatography. The solvent systems are listed in Table III.

Enzyme Studies

NAD was assayed by using the recycling method of Rasmussen, Nielsen, and Schack (60).

Phosphodiesterase (E.C. No. 3.1.4.1) was used in the hydrolysis of 6ANAD to 6ANMN and AMP. See Figure 4. The procedure for hydrolysis followed Kaplan and Stolzenbach's method (61) for production of NMN from NAD except that 6ANAD was substituted for NAD. The products of the 6ANAD reaction and NAD control reaction were separated on a Dowex 1 X 2 column.

NAD glycohydrolase (NADase, E.C. No. 3.2.2.5) from pig brain was used to synthesize 6ANAD from NAD and 6ANam. The procedure of Honjo and Nishizuka (62) was modified to accomplish the exchange reaction. Sixty millimoles of 6ANam and pig brain NADase was used instead of sixty millimoles of NA and beef spleen NADase. The NAD recycling assay measured the loss of NAD. The reaction is shown in Figure 5.

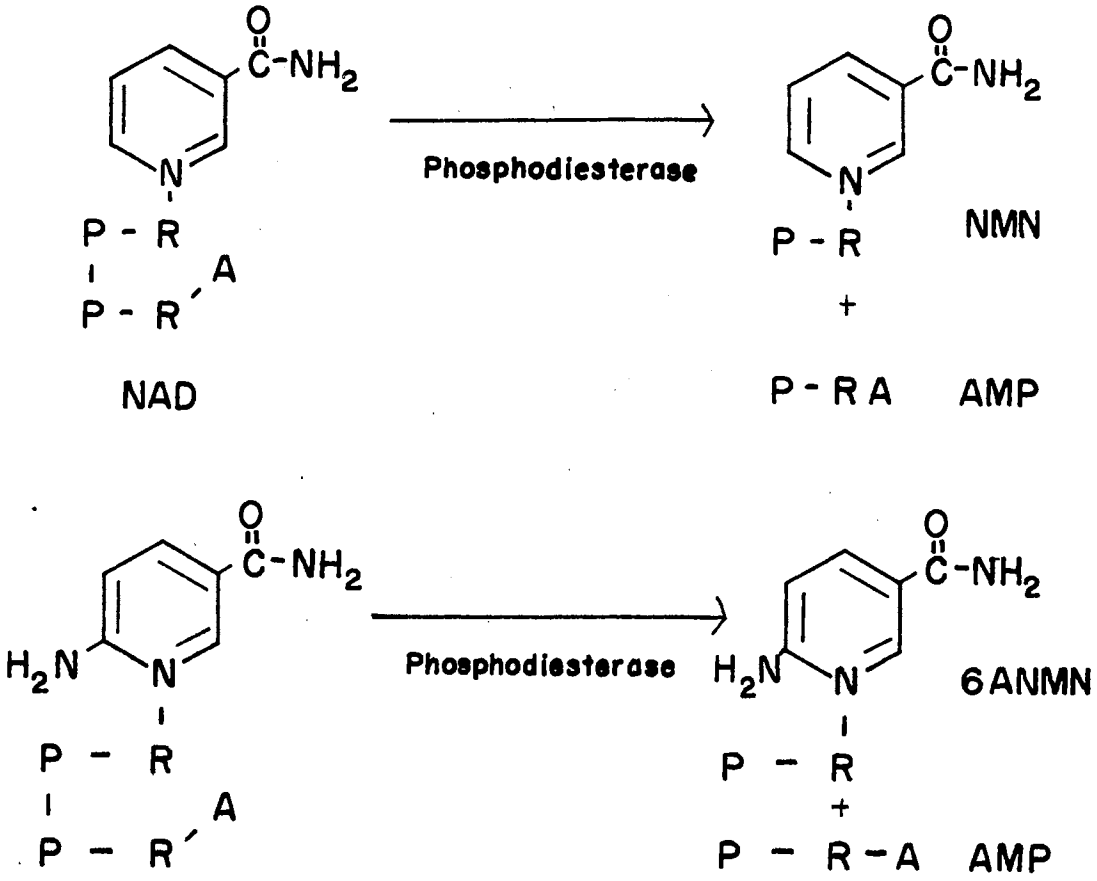
NADase was also used in the identification of 6ANAD produced *in vivo*. One milligram of 6ANAD-^{14}C was added to 0.5 ml of 0.1 M phosphate buffer and 10 milligrams of NADase. At various times 0.1 ml of solution was removed and 0.05 ml perchloric acid added to stop the reaction. The mixture was then analyzed on chromatography system V.

TABLE III

THIN LAYER AND PAPER CHROMATOGRAPHY SYSTEMS

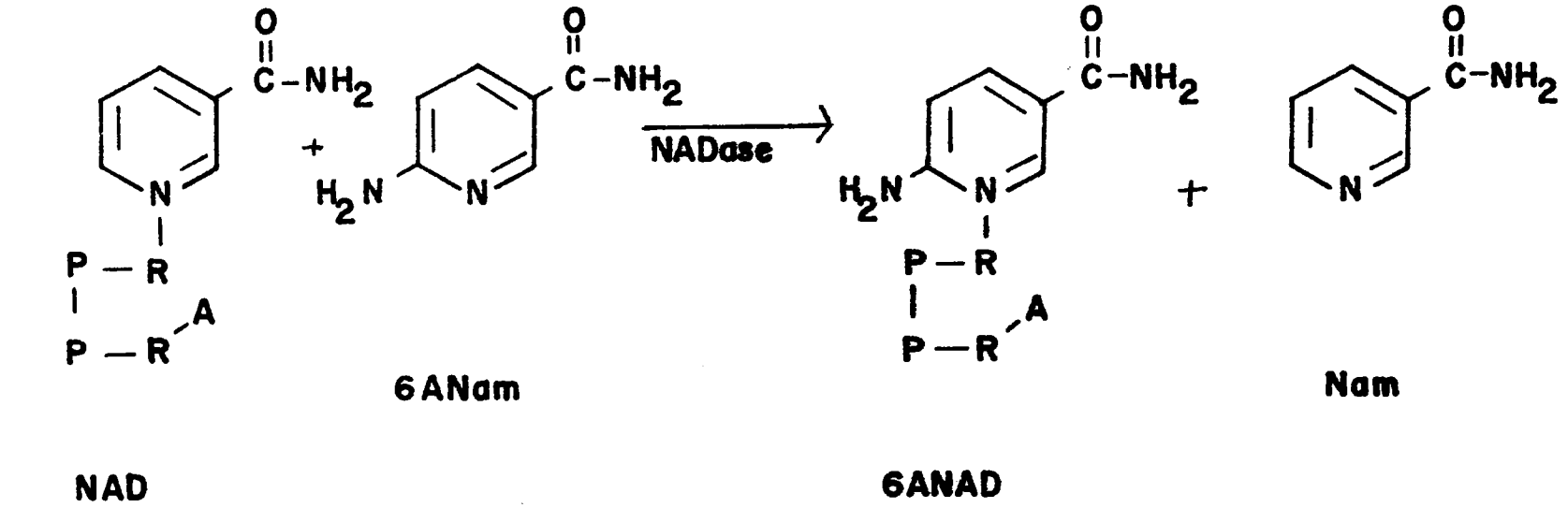
	Support System	Solvent
I	Cellulose plate	n-butanol sat. with H_2O: NH_4,OH, 66:6
II	Cellulose plate	n-butanol sat. with H_2O: acetic acid, 66:3
III	Cellulose plate	isobutyric acid: H_2O: NH_4OH, 66:33:1
IV	Cellulose/PEI	0.1 M LiCl
V	DE81 paper	0.25 M NH_4HCO_3

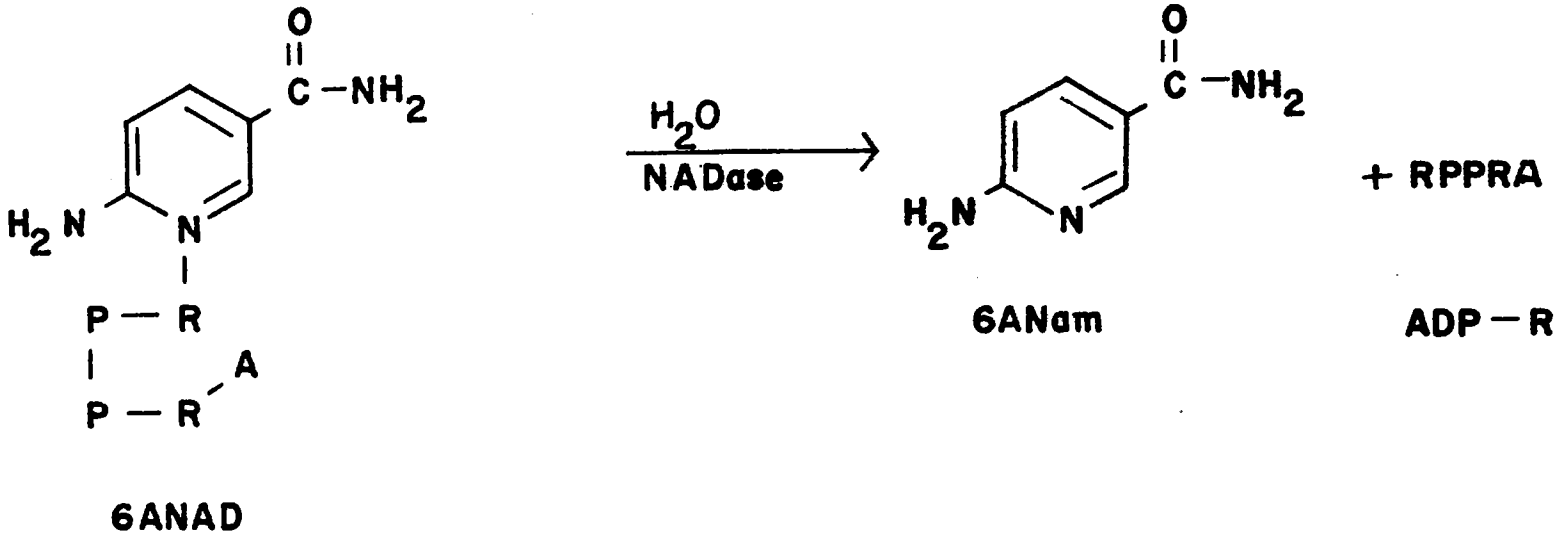
Figure 4. Reactions of Phosphodiesterase



Reaction mixtures contain 0.15 mM pyridine nucleotide, 0.03 M $MgCl_2$, and 100 mg of enzyme in 30 ml of $NaHCO_3$ buffer at pH 8.2.

Figure 5. Reactions of NAD glycohydrolase (NADase). See Text for Experimental Conditions.


NADase
6ANam
Nam
NAD
6ANAD


H2O
NADase
6ANAD
6ANam
+ RPPRA
ADP – R

Chemicals

The $K^{14}CN$ (1mCi/mg) utilized in the synthesis of 6ANA-^{14}C and 6ANam-^{14}C, and the NA-^{14}C were obtained from New England Nuclear. 6ANam, NA, NAm, phosphodiesterase, NAD glycohydrolase, and alcohol dehydrogenase were purchased from the Sigma Company. PABA was purchased from Eastman Organic Chemicals. 2-Amino-5-nitro-pyridine was obtained from Aldrich Company, PPO and POPOP were obtained from the Packard Instrument Company. All other chemicals were obtained from major supply houses.

CHAPTER IV

RESULTS AND DISCUSSION

Conversion of 6ANA-^{14}C to 6ANam-^{14}C

The initial experiment (Figure 6) concerned the conversion of 6ANA to 6ANam in mutant W3899n. Because of the nicotinamide deaminase block, Nam is excreted into the medium when the cells are incubated in the presence of NA. Therefore, if 6ANam is excreted when the bacteria are fed 6ANA, then this analogue must have gone through the pyridine nucleotide cycle. Ten ml cultures of cells were incubated with 2×10^{-4} M 6ANA-^{14}C for six hours. After the cells were removed from the medium, 1 ml each of 10^{-3} M 6ANA and 10^{-3} M 6ANam were added to the medium. The medium was placed on a Dowex 1 formate column and eluted according to the procedure in the Methods. The fractions were monitored for absorbance at A_{260} and ^{14}C content. Two radioactive peaks were observed, Figure 7. These peaks, one eluting with water and the other with formate, coincided with the absorbance peaks of 6ANam and 6ANA respecitvely. Using thin layer chromatrography with systems I and II. For identification, the peak eluting with water was shown to be 6ANam and the peak eluting with formate was 6ANA. The peak eluting with water was also hydrolyzed using 1N NaOH and the product was 6ANA using systems I and II.

Figure 6. The Excretion of Nam-^{14}C from Cells Incubated with NA-^{14}C. See Text for Experimental Details.

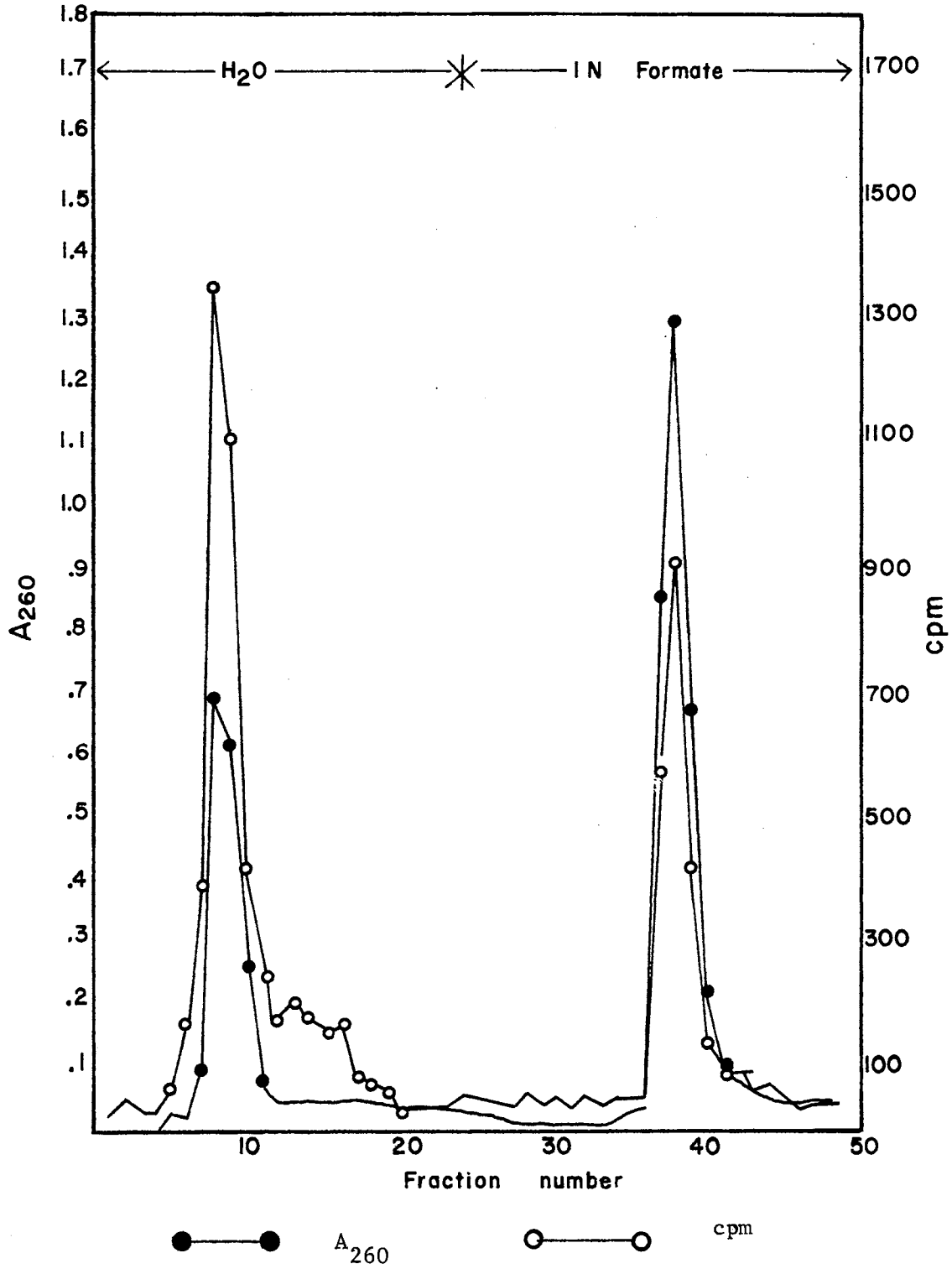
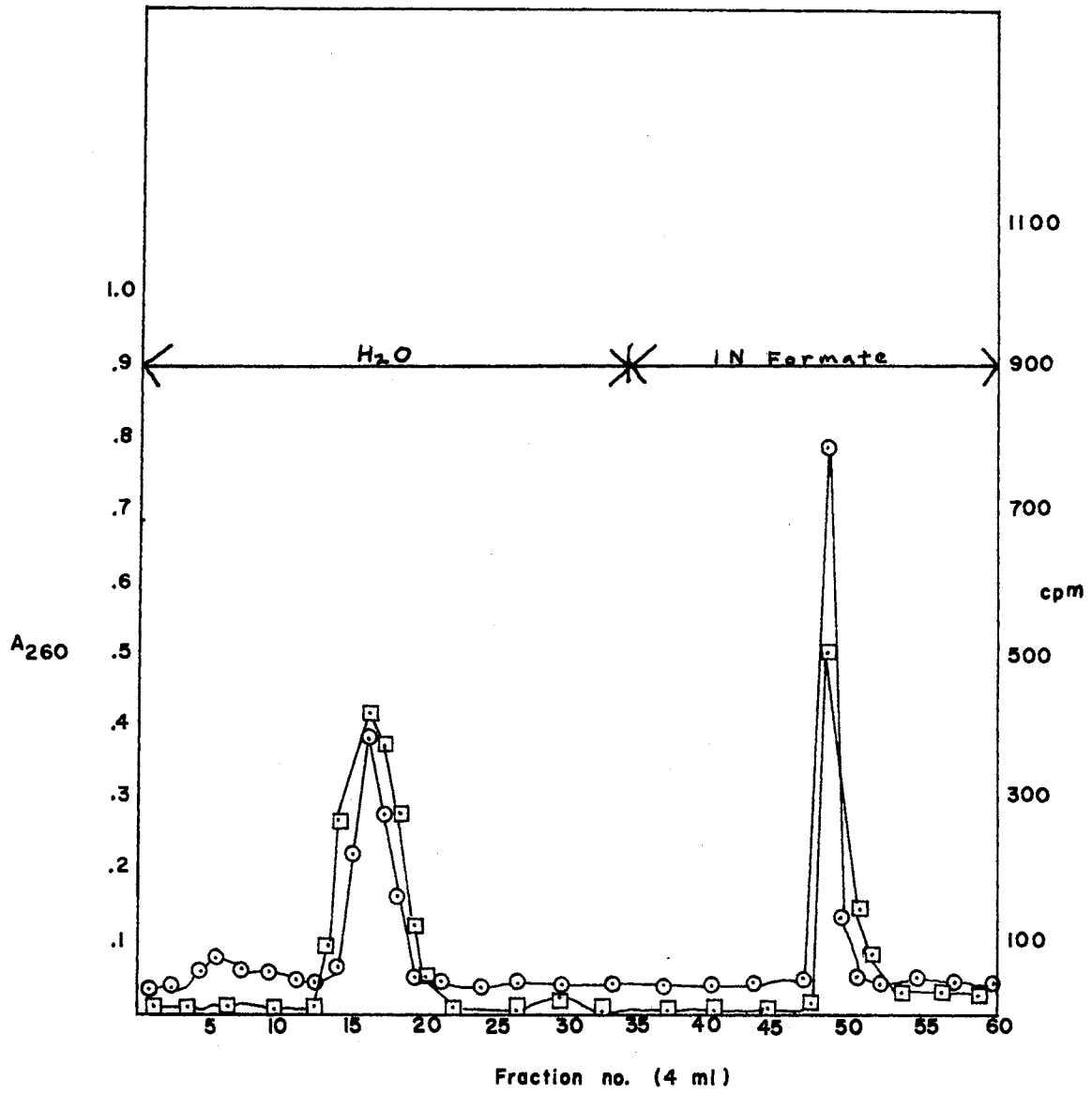

H2O
1 N Formate
A260
cpm
Fraction number
A260
cpm

Figure 7. The Excretion of 6ANam-^{14}C from Cells Incubated with 6ANA-^{14}C.



The medium from E. coli W3899n cells incubated with 2 X 10^{-4}M 6ANA-^{14}C was run on a Dowex 1 X 8 formate column 35cm X 1cm. The column was eluted with H_2O and 1N formic acid. 4 ml fractions were collected.

O-----O = cpm; □-----□ = absorbance at 260nm

Cell Concentration

The concentration of W3899n used in these experiments was 10^9 cells/ml to 4 X 10^9 cells/ml. This optimum range (Figure 8) was determined by incubating the cells at various concentrations and checking for the conversion of 6ANA to 6ANam by ion exchange chromatography. As cell concentration increases the amount of ^{14}C remaining in the medium continuously decreases. However, the maximum excretion of 6ANam-^{14}C occured at a concentration range of 1 X 10^9-2 X 10^9 cells/ml which is the normal concentration of cells in late log phase.

Table IV shows the increase in numbers of cells/ml in the incubation cultures as determined by plate counts. The blank (no NA or 6ANA added) and the 6ANA experiments showed only growth from residual NA present. The NA experiment, however, showed that the W3899n was still in log phase.

Effect of Time Coure on Incubation

The time course of 6ANA-^{14}C uptake (Figure 9) and 6ANam-^{14}C excretion was then determined. W3899n was incubated in a 100 ml culture containing 2 X 10^{-4}M 6ANA-^{14}C. At designated times 1 ml aliquots were removed and centrifuged using an International Clinical centrifuge at 4^{o}. An aliquot of the supernatant (0.1 ml) was counted and 0.1 ml was analyzed by thin layter chromatography system I. The plates were scraped and the scrapings counted for ^{14}C. Initial difference in total dpm in supernat and in 6ANA dpm lies in experimental error.

Figure 8. Determination of Optimum Cell Concentration for Excretion of 6ANam-^{14}C.

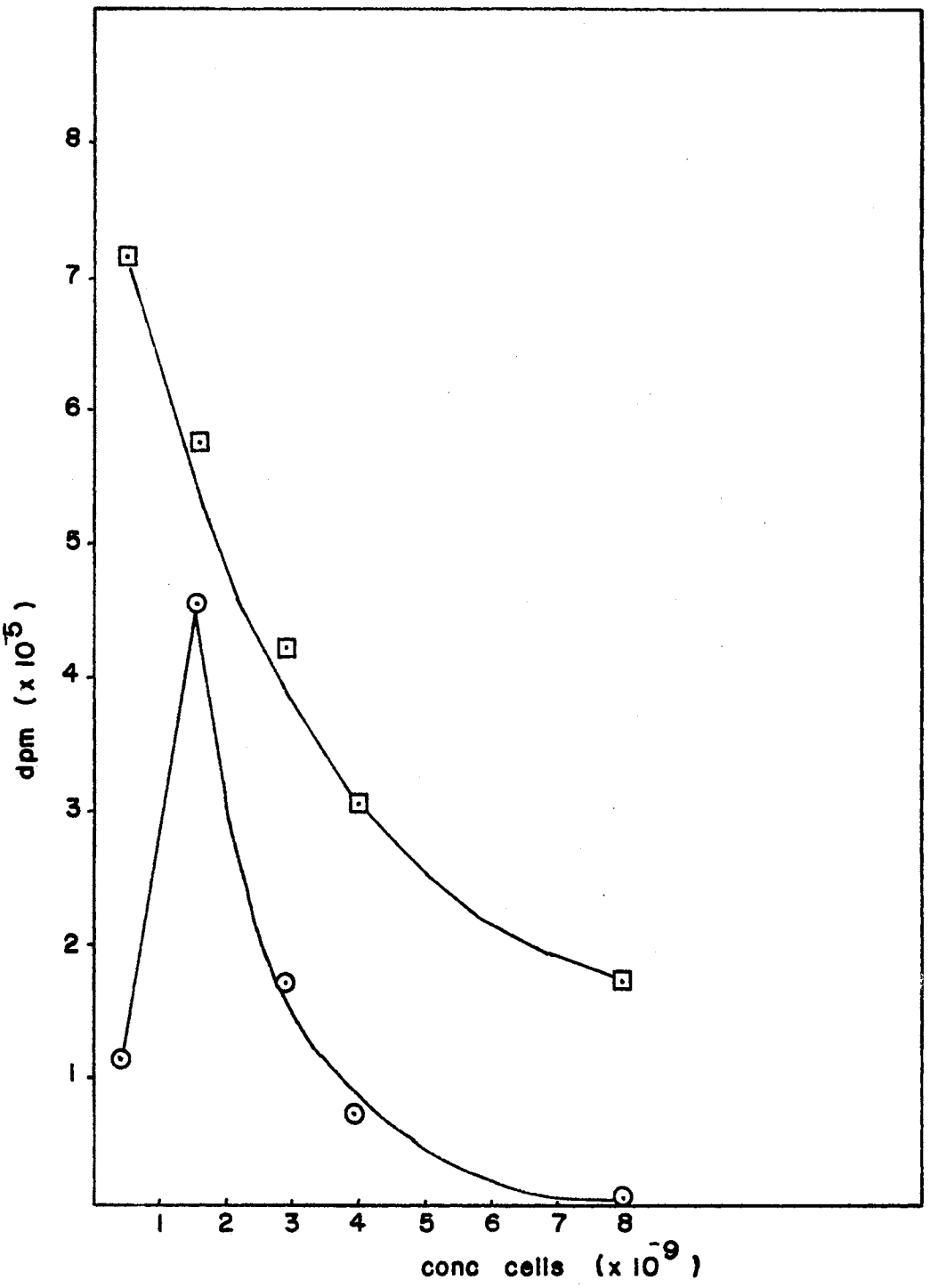

8
7
6
5
4
3
2
1
dpm (x 10^-5)
1 2 3 4 5 6 7 8
conc cells (x 10^-9)
Total dpm in medium
dpm of 6ANam-14C in medium

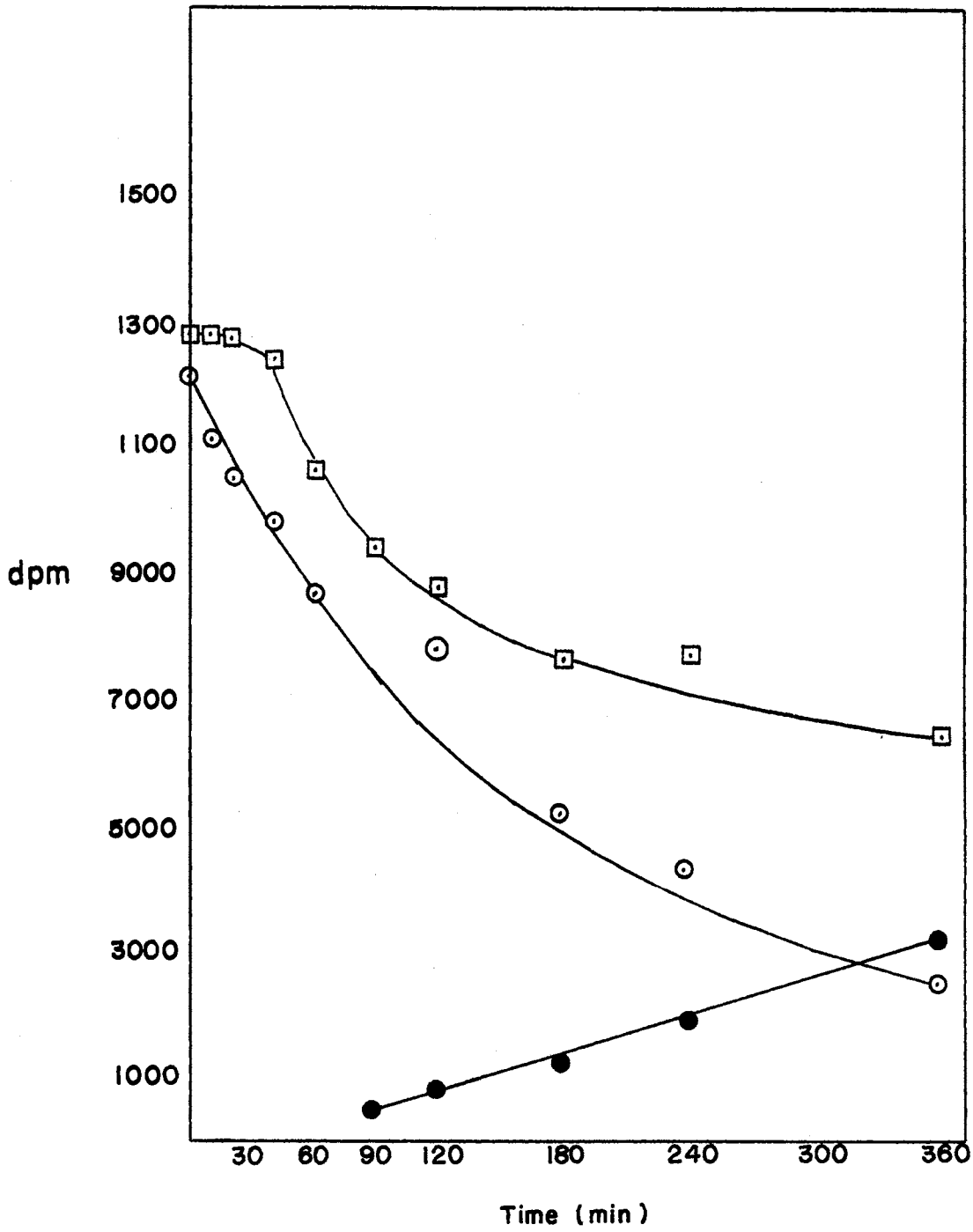
TABLE IV

CELL COUNTS AFTER 6 HOURS OF INCUBATION WITH TEST COMPOUNDS[a]

	cells/ml X 10^9 t=0	cells/ml X 10^9 t=6	t=6 / t=0
Blank	3.3	8.2	2.5
NA	2.7	9.3	3.5
6ANA	2.9	7.7	2.6

[a]See text for experimental details.

Figure 9. Determination of Time Required to Accumulate 6ANA-^{14}C Compounds in Cells



Cells were grown in a 100 ml culture with 2 X 10^{-4}M 6ANA-^{14}C. One ml of media was removed at various times, the cells were removed and the media counted and analyzed on TLC system I.

After an initial lag, the 6ANA-^{14}C is taken up by the cells. After about 90 minutes 6ANam-^{14}C appears in the supernatant but at a slower rate than the disappearance of 6ANA-^{14}C. Therefore, a ^{14}C compound or compounds accumulate inside the cell. After six hours approximately 50% of the ^{14}C is inside the cells. 10% of the ^{14}C was lost presumably as $^{14}CO_2$ or perchloric acid insoluble compounds.

NA-^{14}C, on the other hand, is taken into the cell very rapidly for 90 minutes. At this time the rate of uptake is greatly decreased. See Figure 10. All ^{14}C was recovered in the medium or in the perchloric acid soluble cellular extract.

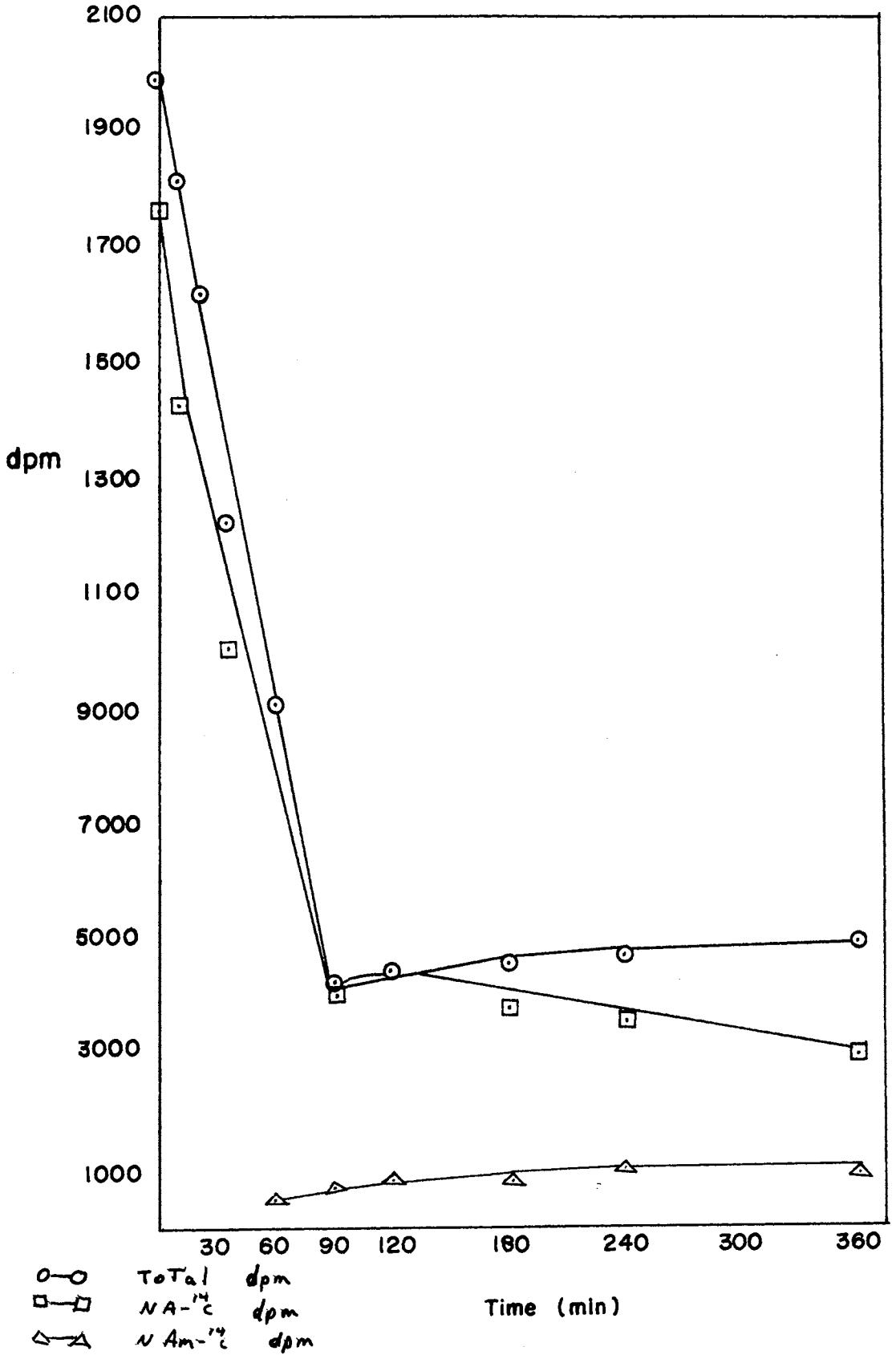
Excretion Controls

Control experiments were run to show that W3899n (nad B pnc A) did not excrete any ^{14}C compounds upon incubation with 6ANam-^{14}C or Nam-^{14}C. In addition to W3899n, W3899 (nad B) was checked. In each case the supernatant was analyzed for the acid and corresponding amide. It was seen that W3899n cannot produce acid from amide while W3899 (nicotinamide deaminase present) can produce acid from amide. Both mutants produce and excrete amide (6ANam-^{14}C and Nam-^{14}C) from acid (6ANA-^{14}C and NA-^{14}C).

Perchloric Acid Soluble Cellular Compounds

Because 6ANA is a structural analogue of paraaminobenzoic acid (PABA), it can be incorporated into a folic acid analogue. To study only the effects of 6ANA on the pyridine nucleotide cycle, PABA was added to the incubation mixture to a concentration of 10^{-3}M to prevent formation of the folic acid analogue of 6ANA. Results of

Figure 10. The Time Course of Uptake of NA-^{14}C and Excretion of Nam-^{14}C. See Text for Experimental Detail.


2100
1900
1700
1500
dpm
1300
1100
9000
7000
5000
3000
1000
30
60
90
120
160
240
300
360
Time (min)
Total dpm
NA-14C dpm
NAm-14C dpm

Dowex-1-Formate chromatography of the perchloric acid soluble cell extract of cells incubated with 6ANA-^{14}C and with/without PABA are shwon in figures 11 and 12 respectively. Only the sixth peak on each profile is changed. Two separate compounds are found with 6ANA-^{14}C and only one with 6ANA-^{14}C and PABA only one.

The perchloric acid soluble fraction of cell extracts from 6ANA-^{14}C plus PABA and NA-^{14}C experiments were also chromatographed on Dowex 1 formate columns. In the case of 6ANA-^{14}C, seven radioactive peaks were observed (Figures 13 & 14). With NA-^{14}C five radioactive peaks were observed. The NA-^{14}C metabolites were identified using cold authenic compounds on ion exchange chromatography, thin layer and paper chromatography Systems I and V. The R_f values are shown in Table V. Peak I is Nam; Peak II is NA; Peak III is NAD; Peak V is NADP; Peak IV is unassigned.

Identification of 6ANAD

Peak V of the 6ANA-^{14}C cell extract contains the highest total activity of the 6ANA-^{14}C metabolites. This peak also contains as detected NAD by the cycling assay and, therefore, was subjected to further investigation. The peak was placed on a DEAE cellulose-HCO_3 column which was eluted with water and then with a NH_4HCO_3 gradient. The radioactive material eluted with water and at the beginning of the gradient. The NAD is eluted from the column (Figure 15) when the conductivity of the elution reaches 2 mmho. The radioactive peak eluting with bicarbonate was further purified over the same column again. Again a small ^{14}C peak was eluted with H_2O and the major ^{14}C peak with (Figure 16). Formation of first peak is evidence that the major peak

Figure 11. Anion Exchange Chromatography of Extract from Cells Incubated with 6ANA-^{14}C and PABA. See Text for Experimental Procedures.

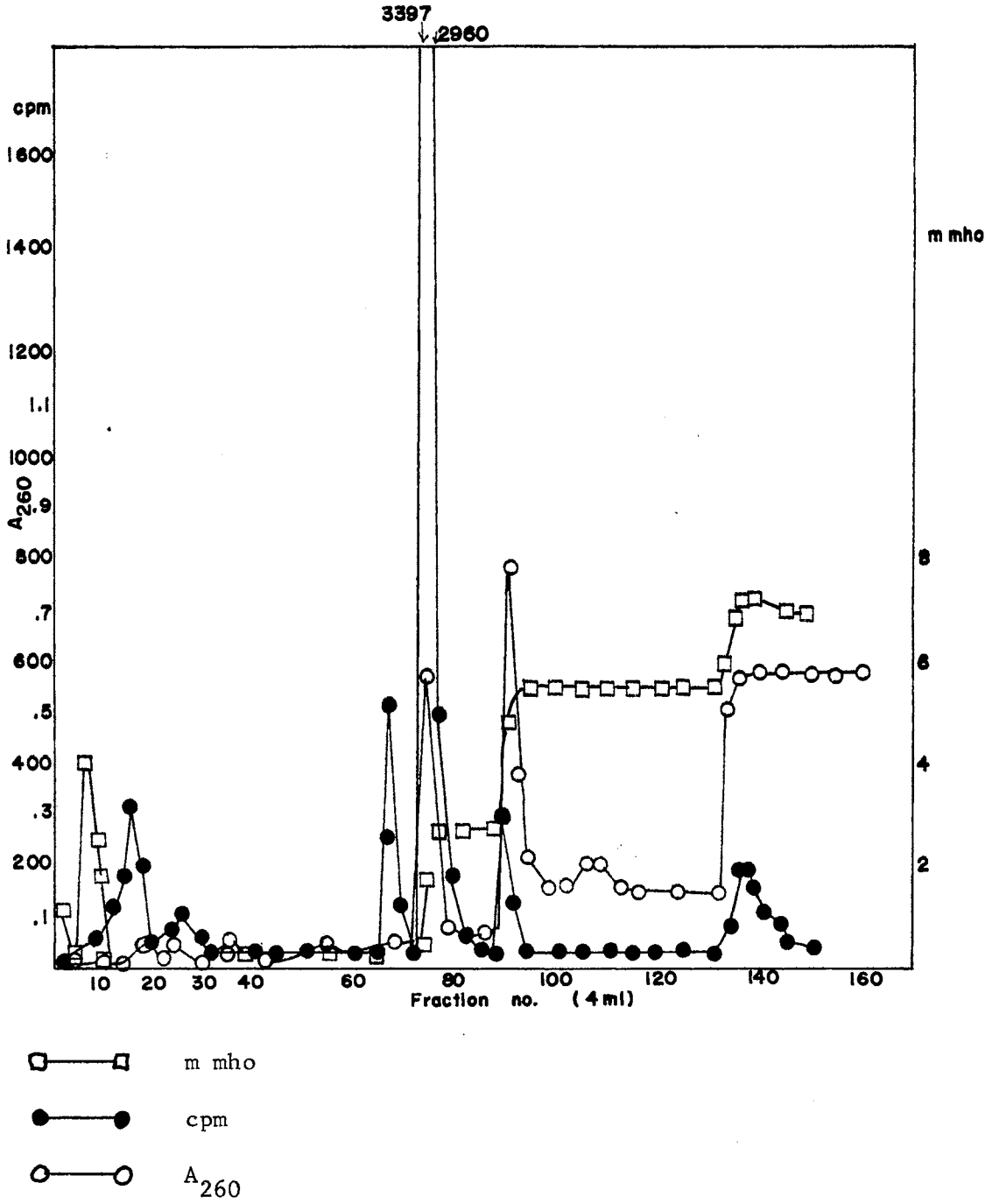

3397
2960
cpm
1600
1400
1200
1.1
1000
A260
.9
800
.7
600
.5
400
.3
200
.1
m mho
8
6
4
2
10
20
30
40
60
80
100
120
140
160
Fraction no. (4 ml)
m mho
cpm
A260

Figure 12. Anion Exchange Chromatography of Extract from Cell Incubated with 6ANA-^{14}C but without PABA. See Text for Experimental Details.

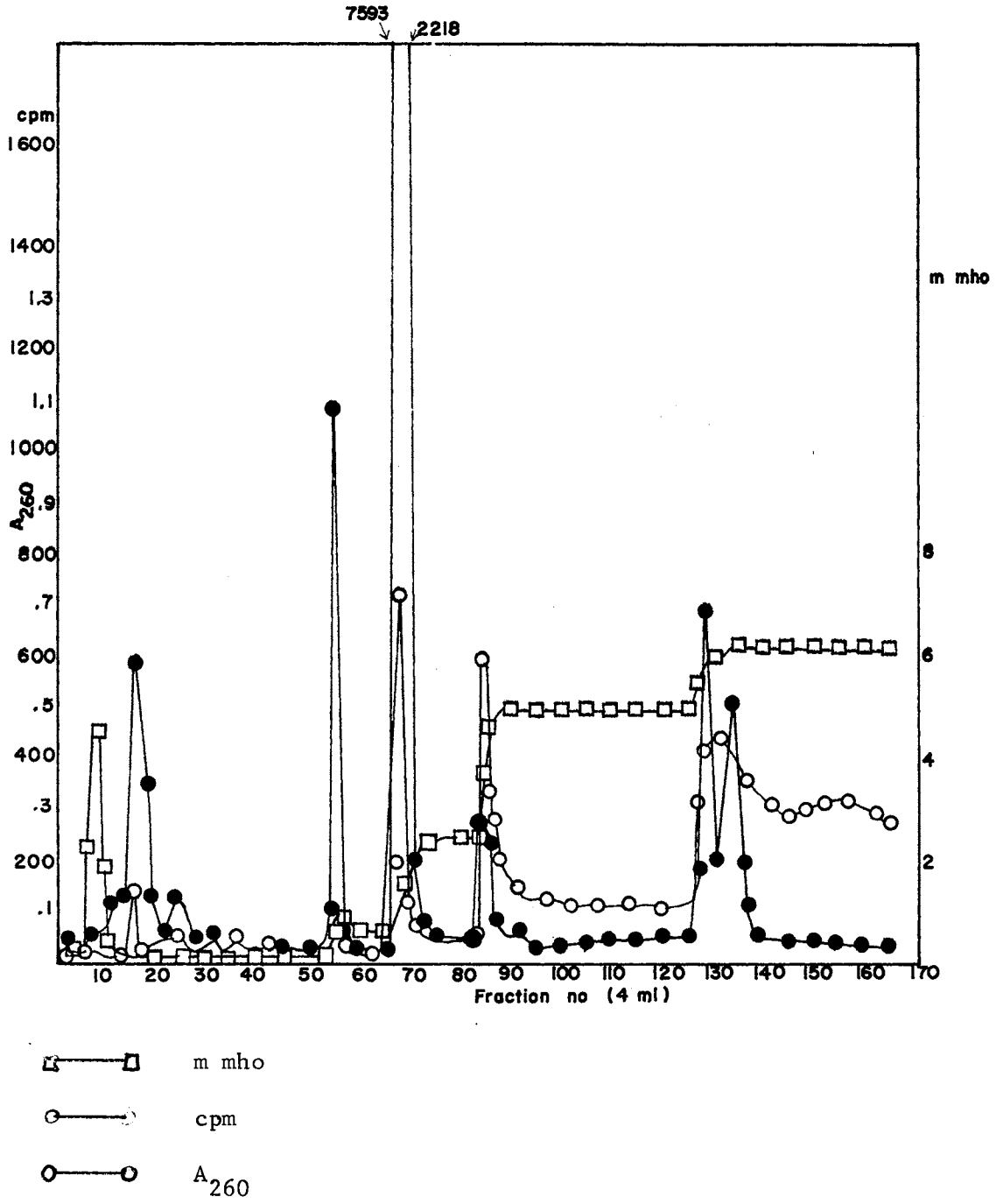

7593
2218
cpm
1600
1400
1200
1000
800
600
400
200
1.3
1.1
.9
.7
.5
.3
.1
A_{260}
m mho
8
6
4
2
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170
Fraction no (4 ml)
m mho
cpm
A_{260}

Figure 13. Anion Exchange Chromatography of Perchloric Acid Soluble Cell Extract from Cells Incubated with 6ANA-^{14}C. See Text for Experimental procedure.

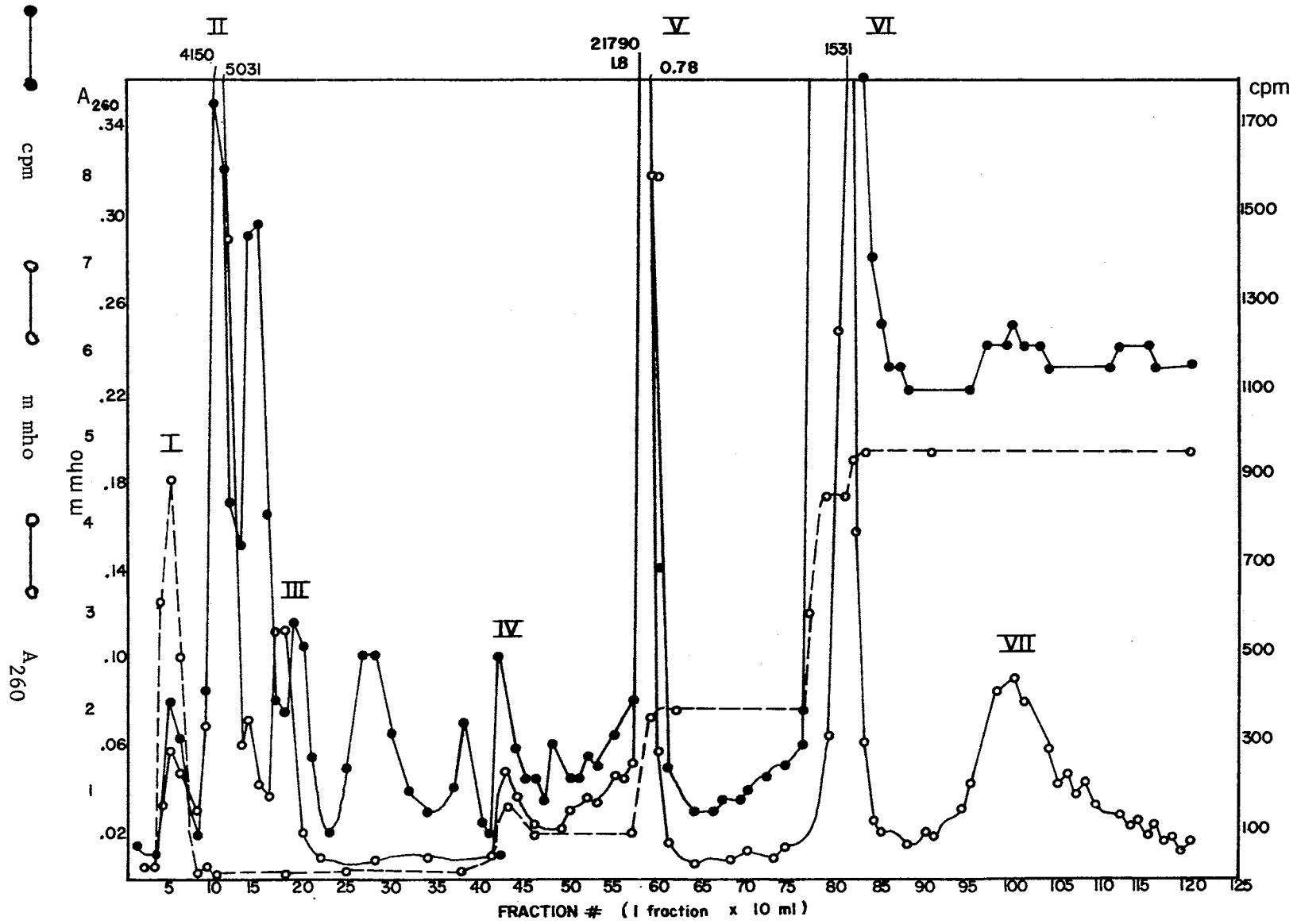

cpm
m mho
A260
II
4150
5031
V
21790
L8
0.78
VI
1531
I
III
IV
VII
A260
.34
.30
.26
.22
.18
.14
.10
.06
.02
mmho
cpm
1700
1500
1300
1100
900
700
500
300
100
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125
FRACTION # (1 fraction x 10 ml)

Figure 14. Anion Exchange Chromatography of Perchloric Acid Soluble Cell Extract from Cells Incubated with NA-^{14}C. See Text for Experimental procedure.

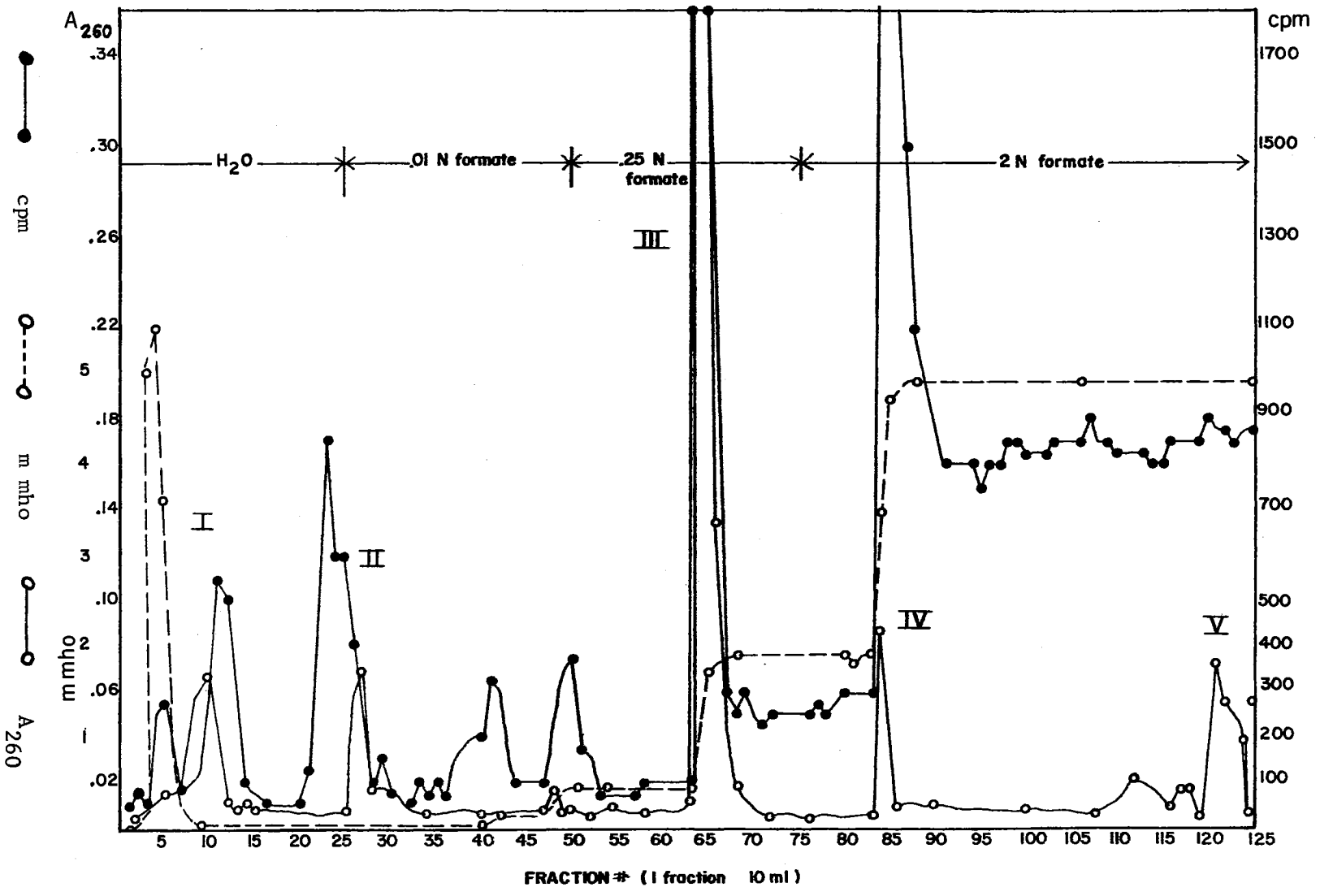

A260
.34
.30
.26
.22
.18
.14
.10
.06
.02
mmho
cpm
1700
1500
1300
1100
900
700
500
400
300
200
100
H2O
.01 N formate
.25 N formate
2 N formate
I
II
III
IV
V
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125
FRACTION # (1 fraction 10 ml)
cpm
m mho
A260

TABLE V

CHROMATOGRAPHY OF NAD-^{14}C COMPOUNDS

Compound	System I	System V
NA	.29	.51
Nam	.78	.76
NMN	.00	.79
NAD	.00	.61
NADP	.00	.27
I	.74	.75
II	.28	.48
III	.00	.57
IV		.52
V	.00	.29

Figure 15. Presumed 6ANAD Analyzed on DEAE Cellulose Column. See Text for Experimental Details.

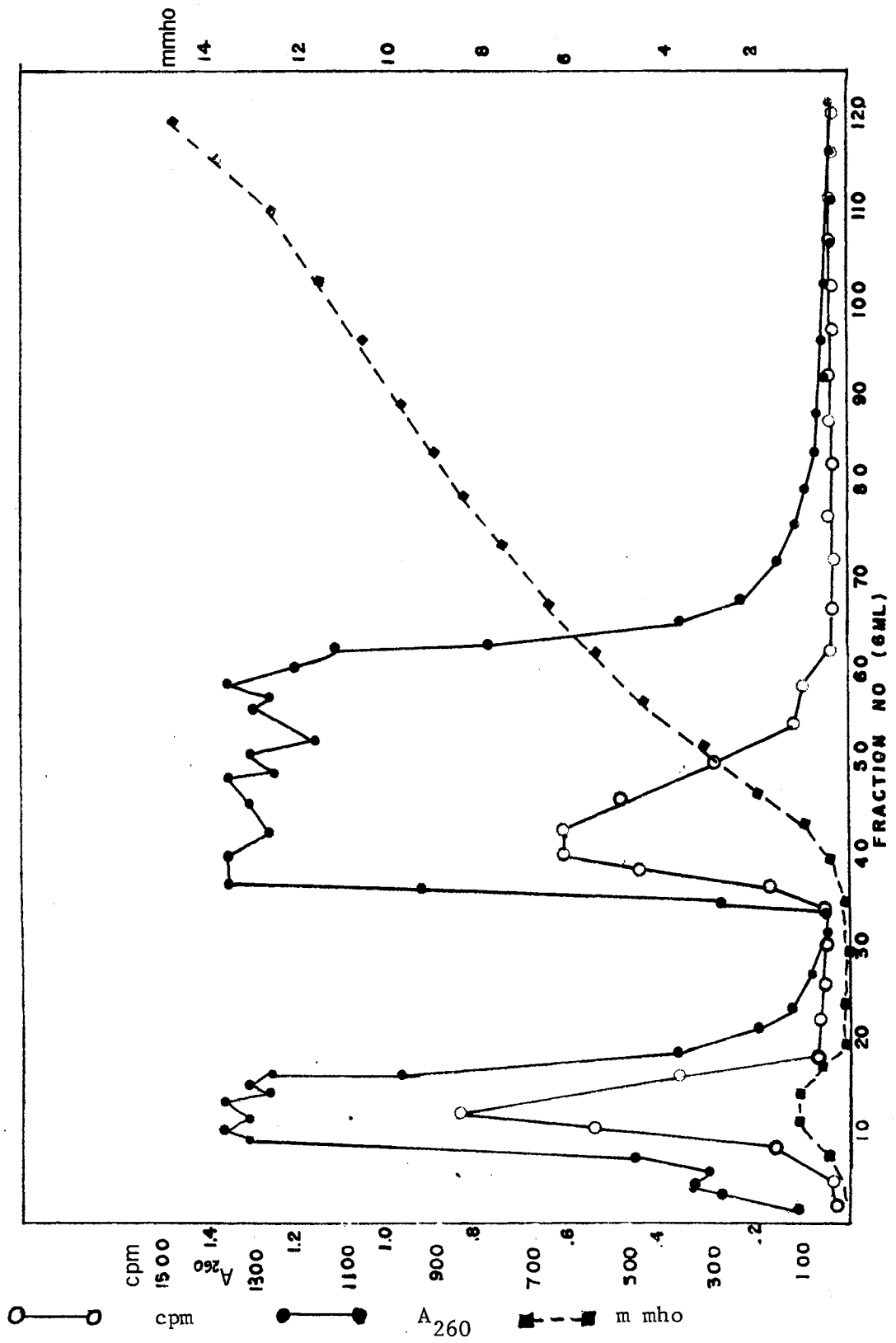
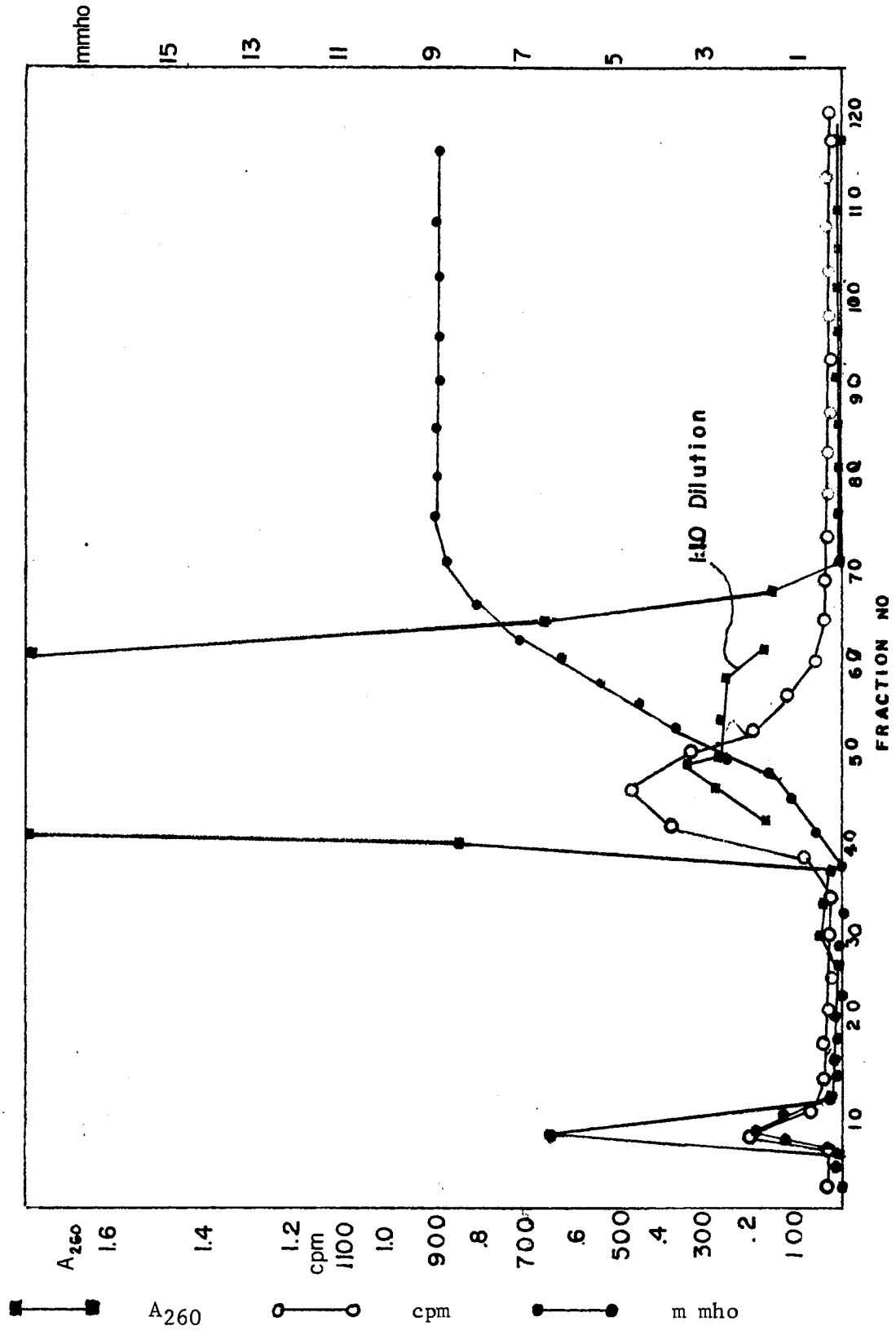

mmho
14
12
10
8
6
4
2
120
110
100
90
80
70
60
50
40
30
20
10
FRACTION NO (6ML)
cpm
1500
1300
1100
900
700
500
300
100
A_{260}
1.4
1.2
1.0
.8
.6
.4
.2
cpm
A_{260}
m mho

Figure 16. Second Purification of 6ANAD on DEAE Cellulose Column. See Text for Experimental Details.


mmho
15
13
11
9
7
5
3
1
1:10 Dilution
FRACTION NO
10
20
30
40
50
60
70
80
90
100
110
120
A260
1.6
1.4
1.2
cpm
1100
1.0
900
.8
700
.6
500
.4
300
.2
100
A260
cpm
m mho

(presumably 6ANAD) breaks down with time.

The peak eluted with bicarbonate was hydrolyzed with snake venom phosphodiesterase and with NADase. The product from the former reaction was analyzed on a 5cm X .2cm Dowex 1 X 2 formate column (Figure 17). The ^{14}C compound was eluted with water and checked in chromatography systems I, II, III, IV, and V, and has a different R_f from the starting material. Se Table VI. The ^{14}C product of the phosphodiesterase reaction is presumed to be 6ANMN.

The NADase ^{14}C reaction products were checked on system V at various times along with authentic 6ANam. The 6ANam has an R_f of 0.50 in this system and the suspected 6ANAD as an R_f of 0.75. NAD has an R_f of 0.74 in this system. From Table VI it is seen that at time 0 the suspected 6ANAD had partially broken down into 6ANam. However, with time the ^{14}C compound with R_f 0.75 disappears from the solution, thus, showing hydrolysis of that compound. This compound is, thus, tenatively identified as 6ANAD.

Other ^{14}C Compounds

When cells of W3899n were incubated in the presence of NA-^{14}C five radioactive peaks were found on Dowex-1 formate chromatography of the cellular extract. Table VII in contrast, seven intracellular radioactive peaks were found after incubating with 6ANA-^{14}C. Most of the compounds formed from 6ANA-^{14}C remain unidentified.

From results obtained with chromatography system I (Table VII) 6ANam-^{14}C is not found as a component of any of the peaks of the cellular extract except as a breakdown product of peak V. The phosphodiesterase product of 6ANAD (6ANMN) was also not observed in the

Figure 17. Chromatography of Phosphodiesterase Hydrolysate on Dowex 1 Formate Column. See Text for Experimental Detail.

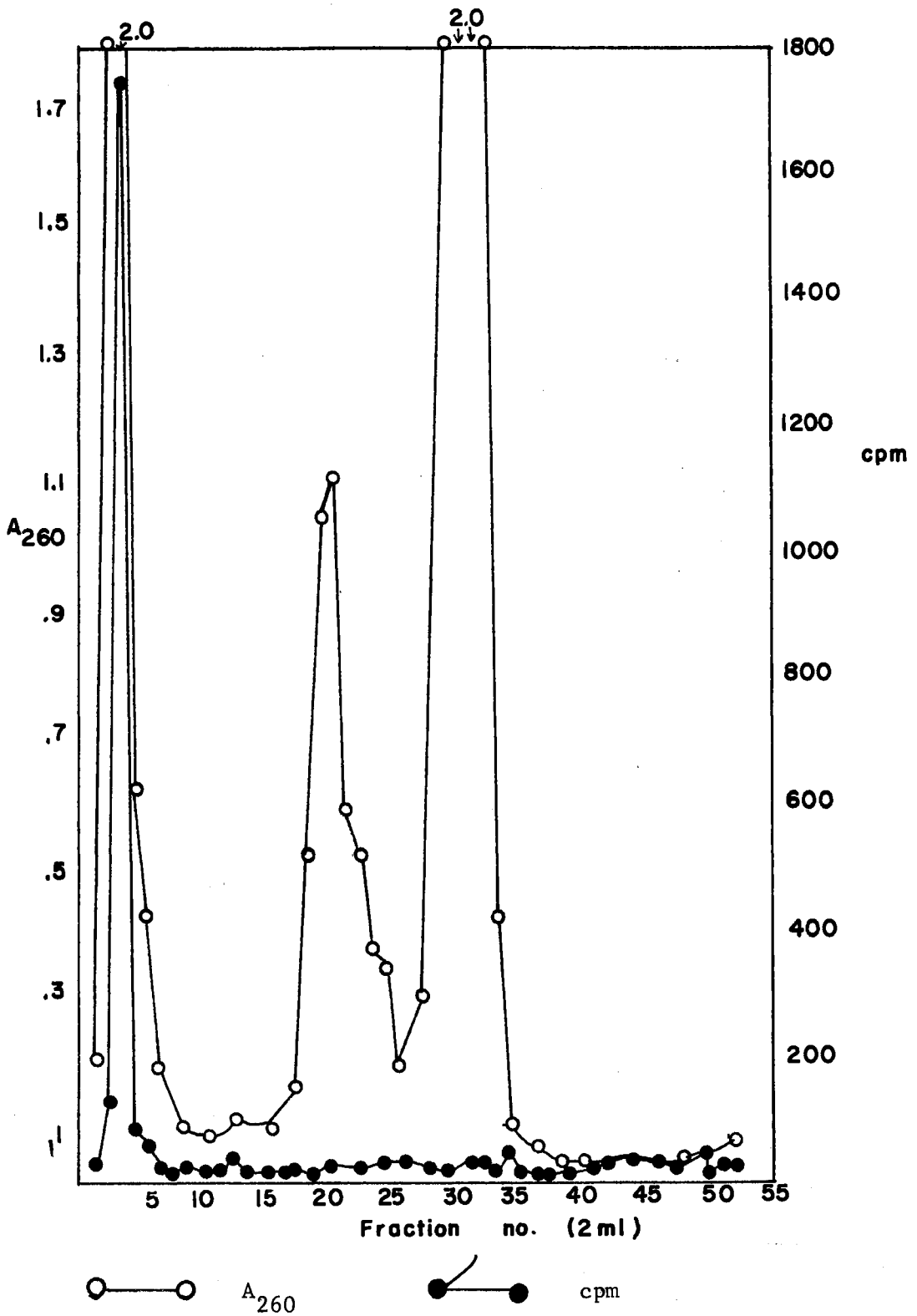

2.0
2.0
1.7
1.5
1.3
1.1
A260
.9
.7
.5
.3
.1
1800
1600
1400
1200
cpm
1000
800
600
400
200
5 10 15 20 25 30 35 40 45 50 55
Fraction no. (2 ml)
A260
cpm

TABLE VI

NADase HYDROLYSIS OF SUSPECTED 6ANAD

	Cpm	
Time	RF .58	RF .71
0	3222	1440
20	2053	1315
40	2660	603
120	2915	235
6ANam	5521	----

TABLE VII

R_f VALUES OF 6ANA-^{14}C METABOLITES ON SEVERAL CHROMATOGRAPHY SYSTEMS

	System				
Peak	I	II	III	IV	V
I	.81		.87	.79	.93
II	.75	.75	.93	.86	.77
III	.38	.75	.80	.07	.80
IV	.25	.75	.80/.27	.43/.86	.43
V	.00	.00	.68/.93	.43	.42/.77
VI	.00	.00		.43	.47
VII	.00			.36/.79	.77/.47
6ANA	.06		.85		
6ANam	.50		.80		.42
6ANAD	.00	.00	.69		.75
Phosphodiesterase Product	.00	.00	.62		.65

cellular extract.

Possibilities for these other peaks are 6ANA riboside, 6ANam riboside, des 6ANAD, 6-amino-nicotinic acid mononucleotide and 6ANADP.

Altered rates of the pyridine nucleotide cycle enzymes with the 6-amino analogues might explain the 2 extra peaks observed with 6ANA-^{14}C. That is the rates of metabolism and breakdown of the analogues could be altered such that a build up of some analogues could occur. Another possibility is that the analogues are much less stable than the natural pyridine nucleotides and break down into several compounds.

CHAPTER V

CONCLUSION

E. coli W3899n converts 6ANA-^{14}C to 6ANam-^{14}C. The excretion of 6ANam-^{14}C is slower than the uptake of 6ANA-^{14}C resulting in an accumulation of ^{14}C compounds in the cell. The conversion of 6ANA to 6ANam suggests that the 6ANA is metabolized through the pyridine nucleotide cycle, since this provides the only know sequency of reactions for converting NA to Namide. A compound isolated from cells incubated with 6ANA treated cells which appears to be the 6-amino analogue of NAD.

These findings support the hypothesis that the bacterostatic action of 6ANA is due to the conversion of 6ANA and 6ANam into pyridine nucleotide analogues which are not functional in oxido-reduction reactions and which repress and/or inhibit the biosynthesis of the normal pyridine nucleotides.

A SELECTED BIBLIOGRAPHY

(1) Florikin, M., and Stotz, E. ad. (1973) Comprehensive Biochemistry, 13, 3rd Ed., Enzyme Nomenclature Recommendations-1972, Elsevier Publishing Co., Inc., New York.

(2) Harden, A., and Young, W. (1909) "The Hexosephosphosphate Formed by Yeast Juice from Hexose and Phosphate." Proc. Roy. Soc., (London) Ser. B 81, 528.

(3) Albus, H., Schlenk, F., and Von Euler, E. (1935) "Uber die CoZymase." Z. Physiol. Chem., 237, 1.

(4) Warburg, O., and Christian, W. (1931) "Uber Aktivierung Der Robisonschen Hexose-Mono-Phosphorsaure in Roten Blutzellen und die Gewinnung Aktivierrenden Fermentlosungen." Biochem. Z., 242, 206.

(5) Wargurg, O., and Christian, W. (1934) "Co-Fermentproblem." Biochem. Z., 274, 112.

(6) Von Euler, H., Schlenk, F., and Vestriu, R. (1937) "Adenosin-Diphosphorsaure aus Cozymase." Naturwissenschaften, 25, 318.

(7) Von Euler, H., Karrer, P., and Bocke, B. (1936) "Charakterisierung von Zuker phosphorsauren und Konstitution der Pentose-Phosphorsaure aus CoZymase." Helv. Chim. Acta., 19, 1060.

(8) Schlenk, F. (1937) "Die Einwirkung von Phosphoroxychlorid auf Cozymase." Naturwissenschaften, 25, 668.

(9) Warburg, O., and Christian, W. (1936) "Pyridin, Der Wassergtoffuber tragende Bestandteil Von Garungsfermenten (Pyridin-Nucleotide)." Biochem. Z., 287, 291.

(10) Warburg, O., and Christian, W. (1935) "Co-Fermentproblem." Biochem. Z., 275, 112 and 446.

(11) Pullman, M. E., San Pietro, A., and Colowick, S. P. (1954) "On the Structure of Reduced Diphosphypyridine Nucleotide, "J. Biol. Chem., 206, 129.

(12) Chandler, J. L. R., Gholson, R. K., and Scott, T. A. (1970) "Studies on the de novo Biosynthesis of NAD in Escherichia coli. I. Labelling Patterns from Precursors." Biochim. Biophys. Acta, 222, 523.

(13) Ortega, M. V., and Brown, G. M. (1960) "Precursors of Nicotinic Acid in Escherichia coli." J. Biol. Chem., 235, 2939.

(14) Ogasawara, N., Chandler, J. L. R., Gholson, R. K., Rosser, R. J., and Andreoli, A. J. (1967) "Biosynthesis of Quinolinic Acid in a Cell-Free System." Biochim. Biophys. Acta, 141, 199.

(15) Andreoli, A. J., Ikeda, M., Nishizuka, Y., and Hayaishi, O. (1963) "Quinolinic Acid: A Precursor to Nicotinamide Adenine Dinucleotide in Eshcherichia coli." Biochem. Biophys. Res. Commun., 12, 92.

(16) Preiss, J., and Handler, P. (1958) "Biosynthesis of Diphosphopyridine Nucleotide. I. Identification of Intermediates." J. Biol. Chem., 233, 488.

(17) Imsande, J., and Pardee, A. B. (1962) "Regulation of Pyridine Nucleotide Biosynthesis in Escherichia coli." J. Biol. Chem., 237, 1305.

(18) Imsande, J. (1961) "Pathway of Diphosphopyridine Nucleotide Biosynthesis in Escherichia coli." J. Biol. Chem., 236, 1494.

(19) Gholson, R. K. (1966) "The Pyridine Nucleotide Cycle." Nature, 212, 933.

(20) Olivera, B. M., and Lehman, I. R. (167) "Diphosphopyridine Nucleotide: A Cofactor for the Polynucleotide-Joining Enqyme From Escherichia coli." Proc. Natl. Acad. Sci. U. S., 57, 1700.

(21) Andreoli, A. J., Okita, T. W., Bloom, R., and Grover, T. A. (1972) "The Pyridine Nucleotide Cycle: Presence of a Nicotinamide Mononucleotide-specific Glycohydrolase in Escherichia coli." Biochem. Biophys. Res. Commun., 49, 264.

(22) Kern, M., and Natale, R. (1958) "A Diphosphypyridine Nucleotidase and Its Protein Inhibitor from Mycobacterium butyricum." J. Biol. Chem., 231, 41.

(23) Swartz, M. N., Kaplan, N. O., and Frech, M. E. (1956) "Significance of 'Heat-Activated' Enzymes." Science, 123, 50.

(24) Manlapaz-Fernandez, P., and Olivera, B. M. (1973) "Pyridine Nucleotide Metabolism in Escherichia coli. IV. Turnover." J. Biol. Chem., 248, 5150.

(25) Tritz, G. J., and Chandler, J. L. R. (1973) "Recognition of a Gene Involved in the Regulation of Nicotinamide Adenine Dinucleotide Biosynthesis." J. Bacteriol., 114, 128.

(26) Tritz, G. J., Matney, T. S., and Gholson, R. K. (1970) "Mapping of the nad B Locus Adjacent to a Previously Undescribed Purine Locus in Escherichia coli K-12." J. Bacteriol., 102, 377.

(27) Tritz, G. J., Matney, T. S., Chandler, J. L. R., and Gholson, R. K. (1970) "Chromosonal Location of the C Gene Involved in Biosynthesis of Nicotinamide Anenine Dinucleotide in Escherichia coli K-12". J. Bacteriol., 104, 45.

(28) Chandler, J. L. R., and Gholson, R. K. (1972) "De Novo Biosynthesis of Nicotinamide Adenine Dinucleotide in Escherichia coli: Excretion of Quinolinic Acid by Mutants Lacking Quinolinate Phosphoribosyl Transerase." J. Bacteriol., 111, 98.

(29) Sundaram, T. K. (1967) "Biosynthesis of Nicotinamide-Adenine Dinucleotide in Escherichia coli." Biochim. Biophys. Acta., 136, 586.

(30) Ruggli, von P., and Staub, A. (1937) "Sereoisomere o-Nitro-und o-Amino-Stilbene, o-Amino-dibenzyl und Ringschluss zu Phenanthren bzw. Dihydro-phenanthren II (o-Amino-dibenzyl (XI). (Mit. O. Schmid)." Helv. Chim. Acta, 20, 50.

(31) Karrer, von P., Schwarzenbach, G., Benz, F., and Solmssen, U. (1936) "Uber Reduktiosprodukte des Nicotinsaure-amid-jodmethylats." Helv. Chim. Acta, 19, 811.

(32) Simpson, M. V. (1954) "Further Studies on the Biosynthesis of Aldolase and Glyceraldehyde-3-Phosphate Dehydrogenase." J. Biol. Chem., 216, 183.

(33) Klenow, H., and Andersen, G. (1957) "Some Enzyme Reaction with Adenine Deoxyriboside Polyphosphates." Biochim. et Biophys. Acta, 23, 92.

(34) Shuster, L., Kaplan, O., and Stolzenbach, F. E. (1955) "The Reaction of Pyridine Nucleotides with Trifluoroacetic Acid Anhydride." J. Biol. Chem., 215, 195.

(35) Zatman, L. J., Kaplan, N. V., and Colowick, S. P. (1953) "Inhibition of Spleen Diphosphopyridine Nucleotide by Nicotinamide, and Exchange Reaction." J. Biol. Chem., 200, 197.

(36) Zatman, L. J., Kaplan, N. O., Colowick, S. P., and Ciotti, M. M. (1953) "Formation of the Isonicotinic Acid Hydrazide Analogue of DPN." J. Am. Chem. Soc., 75, 3293.

(37) Zatman, L. J., Kaplan, N. O., Colowick. S. P., and Ciotti, M. M. (1954) "Effect of Isonicotinic Acid Hydrazide on Diphosphopyridine Nucleotidases." J. Biol. Chem., 209, 453.

(38) Kaplan, N. O. and Ciotti, M. M. (1954) "The 3-Acetyl-Pyridine Analogue of DPN." J. Am. Chem. Soc., 76, 1713.

(39) Kaplan, N. O., and Ciotti, M. M. (1956) "Chemistry and Properties of the 3-Acetylpyridine Analogue of Diphosphopyridine Nucleotide." J. Biol. Chem., 221, 823.

(40) Johnson, W. (1955) "The Inhibition of Sulphanilamide Acetylation by Aromatic and Heterocyclic Carboxamides and Carboxydrazides." Can. J. Biochem. Physio., 33, 107.

(41) Johnson, W. J. (1955) "6-Amino-nicotinamide - a Potent Nicotinamide Antagonist." Science, 122, 834.

(42) Shapiro, D. M., Dietrich, L. S., and Shils, M. E. (1957) "Quantitative Biochemical Differences between Tumor and Host as a Basis for Cancer Chenotherapy. V. Niacin and 6-Amino nicotinamide." Cancer Research, 17, 600.

(43) Martin, D. S., Kligerman, M. M., and Fugmann, R. A. (1958) "Radiotherapy and Adjuvant Combination Chemotherapy (6-Amino nicotinamide and 6-Mercapto purine)." Cancer Research, 18, 893.

(44) Dietrich, L. S., Friedland, I. M., and Kaplan, L. A. (1958) "Pyridine Nucleotide Metabolism: Mechanism of Action of the Niacin Antagonist, 6-Aminonicotinamide." J. Biol. Chem., 233, 964.

(45) Pullman, B., and Pullman, A. (1959) "On the 6-Aminonicotinamide Antagonism of DPN-Dependent Enzymatic Systems." Cancer Research, 19, 337.

(46) Ammon, H. P. T., Patel, I. N., and Steinke, J. (1973) "The Pentose Phosphate Shunt in Glucose-induced Release: In vitro studies with 6-Aminonicotinamide, Methylene, Blue, NAD+, NADH, and nicotinamide of Isolated Pancreatic Rat Islets." Biochim. Biophys. Acta, 297, 352.

(47) Lang, K., Kolbe, H., Keller, K., and Herken, H. (1970) "Der Kohlenhydratstoffwechsel des Gehirns nach Blockade des Pentose-Phosphat-Westes aus 6-Aminonicotinsaureamid." Hoppe-Seylers Z. Physiol. Chem., 351, 1241.

(48) Sullivan, W. T. and Strong, L. M. (1958) "Behavioral Changes in Rats and Guinea Pigs Induced by the Administration of Indole 3-Acetic Acid and 6-Aminonicotinamide." J. Nutrition, 65, 199.

(49) Dietrich, L. S. and Friedland, I. M. (1960) "6-Aminonicotinamide and 6-Aminonicotinic Acid Metabolism in Nucleated and Non-nucleated Erythrocytes." Arch. Biochem. Biophys., 88, 313.

(50) Herken, H., Senft, V., and Zemisch, B. (1954) "Die Einschrankung des tublaren Natrium und Kaliumtransportes durch Biosynthese 6-Amino-nicotinsaureamid enthaltenden Nucleotide." Naunyn. Schmiedebergs Arch. exp. Path. u. Pharmak., 249, 54.

(51) Brunnemann, A. and Coper, H. (1964) "Vergleichende Untersuchungen uber die Biosynthese der NAD(P)-Analogen des 3-Acetylpyridine, 4-Acetylpyridine, 6-Aminonicotinamids und Isonicotinsaurehydrazids" Naunyn-Schmiedebergs Arch. exp. Path. u. Pharmak., 248, 34.

(52) Johnson, W. J., and McColl, J. D. (1955) "Anitmetabolite Activity of 6-Aminonicotinamide." Fed. Proc., 15, 284.

(53) Johnson, O. H., Green, D. E., and Pauli, R. (1943) "The Anti-bacterial Action of Derivatives and Analogues of p-Amino Benzoic Acid." J. Biol. Chem., 153, 37.

(54) Roberts, E. C. and Shealy, Y. F. (1971) "Folic Acid Analogues. Modification in the Benzene-Ring Region. 1. 2'-and 3' -Azafolic Acids." J. Med. Chem., 14, 125.

(55) Wooley, J. G., Murphy, M. K., Bond, H. W., and Perrine, T. D. (1952) "The Effect of Certain Chemical Compounds on the Multiplication of T_2 Bacteriophage." J. Immun., 68, 523.

(56) Wacker, V. A., Zochmann, E. R., and Trager, L. (1967) "Biosynthese Folsaure-und NAD-analoges Verbindungen." Hoppe-Seylers Z. Physiol. Chem., 348, 455.

(57) Schwartz/Mann-SRB Technical Brochure 64D1 Revised: July, 1969.

(58) Yates, R. A., and Pardee, A. B. (1956) "Pyrimidine Biosynthesis in Escherichia coli." J. Biol. Chem., 221, 743.

(59) Wang, C. H. and Willis, D. L. (1965) "Measurement of Radioactivity by the Liquird (Internal Sample) Scintilliation Method," Radio-tracer Methodology in Biological Science, Prentice-Hall, Inc., Englewood Cliffs, N. J., p. 104.

(60) Rasmussen, H. N., Nielsen, J. R., and Schack, P. (1972) "Simple and Sensitive Photometric Recycling Assay for Nicotinamide-Adenine Dinucleotide." Anal. Biochem., 50, 642.

(61) Kaplan, N. O., and Stolzenbach, F. E. (1957) "Preparation of DPN Derivatives and Analogues." Methods in Enzymology, vol. 3, S. P. Colowick, and N. O. Kaplan, Eds., Academic Press, N. Y. p. 899.

(62) Honjo, T., and Nishizuka, Y. (1971) "Enzymatic Preparation of Nicotinic Acid Adenine Dinucleotide, Nicotinic Acid Ribonucleotide, and Nicotinic Acid Ribonucleotide," in Methods in Enzymology, S. P. Colowick, and N. O. Kaplan, Eds. Academic Press, N. Y., p. 132.

VITA

John Ronald Cobb

Candidate for the Degree of

Master of Science

Thesis: THE MECHANISM OF ACTION AND EFFECTS OF THE NIACIN ANALOGUE 6-AMINONICOTINIC ACID IN ESCHERICHIA COLI

Major Field: Biochemistry

Biographical:

Personal Data: Born in Washington D. C., December 2, 1949, the son of Mr. and Mrs. H. L. Cobb, Jr. Married to Beverly Shartel Cobb, Father to one child, Amy Rebecca.

Education: Graduated from East Central High School, Tulsa, Oklahoma, in May, 1968; received Bachelor of Science degree in Biochemistry from Oklahoma State University in May, 1972; completed requirements for the Master of Science degree at Oklahoma State University in July, 1974.

Professional Experience: Laboratory technician, Department of Biochemistry, Oklahoma State University, January, 1972 to September, 1972; Graduate Research Assistant, Department of Biochemistry, Oklahoma State University, September, 1972 to July, 1974.