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The requirement of niacin and its biosynthesis from tryptophan in the nematode, *Caenorhabditis elegans*

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**The requirement of niacin and its biosynthesis from tryptophan
in the nematode, *Caenorhabditis elegans****

Li, Chih-Ping, M.S.

San Jose State University, 1994

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THE REQUIREMENT OF NIACIN AND ITS BIOSYNTHESIS FROM
TRYPTOPHAN IN THE NEMATODE, Caenorhabditis elegans

A Thesis

Presented to

The Faculty of the Department of Nutrition and Food science
San Jose State University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
in Nutritional Science

By

Chih-Ping Li

May, 1994

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Chih-Ping Li

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APPROVED FOR THE DEPARTMENT
OF NUTRITION AND FOOD SCIENCE

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ABSTRACT

THE REQUIREMENT OF NIACIN AND ITS BIOSYNTHESIS FROM TRYPTOPHAN IN THE NEMATODE, Caenorhabditis elegans

by Chih-Ping Li

The requirement for niacin was determined in the free-living nematode, Caenorhabditis elegans (C. elegans). The growth-promoting activity of 0 (control), 0.060, 0.30, 1.5, 7.5, 38, 190, and 940 $\mu\text{g/ml}$ of nicotinic acid (N), nicotinamide (Nm), and nicotinic acid plus nicotinamide (N + Nm) was quantitatively determined. The deficient, optimal, and toxic levels for N or Nm were below 1.5, between 1.5 to 190, and above 190 $\mu\text{g/ml}$, respectively. Nicotinic acid (N) demonstrated greater growth-promoting activity than Nm at all levels. The deficient, optimal, and toxic levels for N + Nm were below 0.30, between 0.30 to 38, and above 38 $\mu\text{g/ml}$, respectively. In studying the role of tryptophan as a niacin precursor, the basal medium (containing 0.30 $\mu\text{g/ml}$ of N and 180 $\mu\text{g/ml}$ of tryptophan) was supplemented with additional N (0, 0.60, or 1.2 $\mu\text{g/ml}$) or additional tryptophan (0, 180, 370, 550, 740, or 920 $\mu\text{g/ml}$). Using Finney's slope ratio procedure, the conversion ratio of tryptophan to niacin was determined to be 500:1 in C. elegans.

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PREFACE

The following is a publication style thesis. Chapter 2 is a journal article written according to guidelines published by the Journal of Nutrition. Chapters 1 and 3 are written according to guidelines outlined in the Publication Manual of the American Psychological Association (3rd. edition), 1990.

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CHAPTER 1
INTRODUCTION AND REVIEW OF LITERATURE

Introduction

Caenorhabditis elegans (C. elegans) is a free living nematode that can be cultivated under axenic (germ-free) conditions. This nematode has a short life-cycle (34 days) (Croll & Mathews, 1977), a rapid generation time (3.5 days) and the ability to be cultivated in large quantities for bioassay under defined and controlled conditions (Marx, 1984). Therefore, it has become widely used as a model for genetic (Kenyon, 1988), biological (Zuckerman, 1981), and nutritional (Lu et al., 1983; Bolla, 1987; Lu & Goetsch, 1993) research. In 1962, Nicholas et al. reported that C. elegans failed to reproduce if niacin was omitted from the medium. However, the quantitative requirement of niacin necessary for optimal growth was not determined.

Niacin is a collective term that includes nicotinic acid and nicotinamide, both natural forms of the vitamin with niacin activity (Nomenclature Policy, 1979). Tryptophan, a precursor for niacin in animals, also contributes to niacin nutriture. This was first demonstrated in experiments with rats that were fed diets high in corn and low in niacin by Krehl and his coworkers

(1945). They found that either niacin or tryptophan overcame the dietary deficiency. In humans, it is estimated that 60 mg tryptophan can be converted to 1 mg niacin (Goldsmith et al., 1961).

The objectives of this study were: 1) to quantitatively determine the niacin requirement for optimal population growth of the nematode, C. elegans; 2) to compare the growth-promoting activities of the two forms of niacin, nicotinic acid and nicotinamide; and 3) to determine the efficiency of tryptophan as a niacin precursor in C. elegans.

Review of Literature

The Nematode, C. elegans

Biological Characteristics

Caenorhabditis elegans (C. elegans) is a free-living microscopic nematode, which has been cultivated under germ-free conditions (Riddle, 1982). Since it is a species consisting almost entirely of hermaphroditic, self-fertilizing individuals (Brun, 1965); a large population (approximately 90,000 nematodes per ml of medium) can be cloned from a single hermaphrodite for bioassay. Therefore, C. elegans has become widely used as an animal model for genetic (Kenyon, 1988), biological (Zuckerman, 1981), and nutritional (Lu et al., 1983; Bolla, 1987; Lu & Goetsch,

1993) research.

C. elegans is cylindrically shaped and tapered at both ends (Figure A). Adult nematodes of C. elegans are small (about 1 millimeter in length and 0.1 millimeter in width), and consist of approximately 1000 somatic cells (Marx, 1984). This species has a short life cycle (34 days) (Croll & Mathews, 1977); and the fertilized egg develops into a mature animal in only 3.5 days (Marx, 1984). Normally, the life cycle of C. elegans consists of six stages: the egg, four larval stages, and the adult. The larva progresses through a series of four molts to adulthood (Kenyon, 1988). In the absence of food, however, the second molt produces a dauer larva which has an altered cuticle and can withstand adverse conditions. When presented with food, the dauer larva molts and continues the normal progress toward adulthood (Riddle, 1982).

The growth and reproduction of C. elegans are very sensitive to temperature. The optimal temperature is 18° to 22°C (Croll & Mathews, 1977). At 18°C, C. elegans shows an average fecundity of 141 offspring per hermaphrodite (Fatt and Dougherty, 1963). When the growth conditions are changed from 18°C to 23°C, the resulting adult has a very low fecundity. If the succeeding generations are kept at 23°C, fecundity decreases, and complete sterility is reached by the fifth or sixth generation (Brun, 1965). Thus there

Figure A. Anatomy of *C. elegans*.
 Note. From "Caenorhabditis elegans: Getting to know you" by
 J.L. Marx, 1984, *Science*, 225, p. 40.

exists between normal (around 20°C) and immediately sterilizing (above 24°C) temperatures, a range that leads to the extinction of the strain within a few generations.

The nutritional requirements of C. elegans and C. briggsae have been extensively studied. These two species of nematodes are closely related to each other. They differ only by genetic composition and the ability to interbreed (Friedman et al., 1977; Nicholas, 1984). Some papers published on C. briggsae may in fact describe work on C. elegans (Nicholas 1984).

Nutritional requirements

Vitamins. Nicholas et al. (1962) reported that thiamin, riboflavin, folic acid, niacin, pantothenic acid, and pyridoxine are required for the normal growth and reproduction of C. briggsae. C. elegans exhibits similar vitamin requirements. C. elegans requires a minimal thiamin concentration of 0.075 µg/ml of culture media for optimal population growth (Augustin, 1992). Pyridoxamine, pyridoxine, and pyridoxal phosphate (vitamin B₆) promote growth in C. elegans (Sun et al., 1986). In vitamin B₆-deficient nematodes, tryptophan metabolites (xanthurenic and kynurenic acids) accumulate in the culture medium during the tryptophan-load test. These studies indicate that C. elegans requires vitamin B₆.

The folic acid, vitamin B₁₂, and biotin requirements of

C. elegans have also been assessed. In 1974, Lu et al. found that the accumulation of formimino-L-glutamic acid (FIGLU) in nematode tissues is related to folic acid deficiency. It was suggested that folic acid is required for the catabolism of histidine in nematodes. Lu et al. (1976) reported that the biosynthesis of methionine from homocysteine requires both vitamin B₁₂ and folic acid. A biotin requirement was demonstrated by adding avidin, a protein that inhibits the uptake of biotin, to the culture medium (Nicholas & Jantunen, 1963).

Minerals. The quantities of magnesium, sodium, potassium, manganese, calcium, and copper required by C. elegans were determined by Lu and coworkers in 1983. An individual mineral deficiency was developed by deleting the mineral from the basal medium. The quantitative requirements for each mineral were determined to be 73, 300, 530, 6.3, 1500, and 7.2 µg/ml, respectively. Zinc is also an essential mineral for C. elegans. Weber (1992) found that a level of 4.9 to 37 µg/ml zinc in the media supported optimal growth in C. elegans.

Amino acids. The amino acid requirements of C. briggsae were investigated by Vanfleteren (1973). Arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine are not synthesized by nematodes at levels high enough to permit

reproduction; they are referred to as essential amino acids. On the other hand, alanine, asparagine, cysteine, glutamate, glutamine, glycine, proline, serine, and tyrosine, are synthesized by *C. elegans*; they are referred to as nonessential amino acids.

Other growth factors. In 1968, Hieb and Rothstein showed that *C. briggsae* requires sterol. The sterol requirement of *C. elegans* can be satisfied by a single sterol or a mixture of sterol compounds. Media containing ergosterol, 7-dehydrocholesterol, β -sitosterol, or stigmasterol support rapid reproduction. Population growth was comparable to that obtained with a mixture of sterols. In 1977, Lu et al. found the optimal level of β -sitosterol required by *C. elegans* to be 50 μ g/ml. *C. briggsae* requires heme (an iron-containing porphyrin compound). Hieb et al. (1970) demonstrated that pure myoglobin, hemoglobin, cytochrome c, and hemin can substitute effectively for the liver fraction in the medium. Cytochrome c is the most effective heme source for promoting growth and reproduction in *C. briggsae*, and its functions are similar to that of myoglobin in the higher animals.

Energy source. The energy requirement of *C. elegans* can be met by either potassium acetate or glucose. In 1978, Lu et al. reported that potassium acetate (5 mg/ml) in media containing a minimal amount of glucose (1.3 mg/ml) can

be used as an energy source by nematodes. Fatty acids can also be used as an energy source because fatty acids can be metabolized to acetate by β -oxidation. The fatty acid oleate (or stearate) was more active for population growth in C. briggsae, while linoleate was less active on both a weight and molar basis. Furthermore, Lu et al. (1978) reported that other lipid-related compounds (Tween 80, Tween 85, ethanol, and n-propanol) can greatly stimulate population growth in C. briggsae in place of undefined proteinaceous sources, e.g. casamino acids. In fact, these lipid-related compounds are even more potent than casamino acids in the basal medium.

Hansen and Buecher (1970) found that reproduction of C. elegans was impaired by the omission of glucose and trehalose from basal medium. Lu and Goetsch (1993) reported that C. elegans can utilize carbohydrates, especially glucose as a very effective energy sources. Their results indicate that a glucose concentration of 32.5 mg/ml should be used to replace the concentration of 1.3 mg/ml originally used in the defined medium for optimal growth of C. elegans. With the increased level of glucose, potassium acetate is no longer needed in the medium.

Culture Conditions

In 1962, Tomlinson and Rothstein reported that cultivation of free-living nematodes under axenic (germ-

free) conditions required a semi-defined medium. This medium was composed of two parts, a chemically defined portion and a small quantity of undefined materials. The most satisfactory chemically defined medium previously reported is Caenorhabditis briggsae Maintenance Medium (CbMM) (Buecher et al., 1966). CbMM is an extremely rich medium composed of pre-determined amounts of amino acids, vitamins, nucleic acids, minerals, glutathione, glucose, and other growth factors. The undefined materials are obtained from mammalian liver, chick embryo, and/or bacteria (Nicholas & Jantunen, 1963). A variety of proteinaceous sources have also been used to provide unidentified growth factors, including commercially available products such as soy peptone (Lu et al., 1976), egg albumin (Buecher et al., 1966), and casamino acids (Lu et al., 1974). More recently, C. elegans has been cultivated axenically in a chemically defined medium consisting of CbMM and three additional defined compounds: β -sitosterol (sterol source) (Hieb & Rothstein, 1968; Lu et al., 1977), cytochrome c (heme source) (Hieb et al., 1970), and potassium acetate (energy source) (Lu et al., 1978). In 1993, Lu and Goetsch demonstrated that glucose at 32.5 mg/ml (instead of 1.3 mg/ml in the CbMM) can completely replace potassium acetate as the energy source in the nematode. Based on this study, the chemically defined medium used for cultivation of C.

elegans has been modified to: CbMM containing 32.5 mg/ml glucose, 50 µg/ml cytochrome c and 50 µg/ml β-sitosterol. The newly developed medium consists of 55 compounds and is named C. elegans Maintenance Medium (CeMM) (Appendix I) (Lu & Goetsch, 1993).

Niacin

Niacin (Figure B) is a collective term for two forms of the vitamin with niacin activity: nicotinic acid and nicotinamide. The term "niacin" is used as a generic descriptor of pyridine 3-carboxylic acid and derivatives that exhibit qualitatively the biological activity of nicotinic acid and nicotinamide (Nomenclature Policy, 1979).

The Function of Niacin

The coenzyme forms of niacin are nicotinamide adenine nucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) (Figure B). Hundreds of enzymes require the nicotinamide moiety (NAD or NADP) as coenzymes. The most important function of the coenzymes is to help bring about the action of the enzymes known as dehydrogenases, which are essential in oxidation-reduction reactions and catalyze such diverse reactions as the conversion of alcohols (often sugars and polyols) to aldehydes or ketones, hemiacetals to lactones, aldehydes to acids, and certain amino acids to keto acids (Snell, 1953). The mechanism of

Vitamins

Nicotinic acid

Nicotinamide

Coenzymes

NAD and NADP

Figure B. Structures of the vitamins nicotinic acid and nicotinamide and the coenzymes NAD and NADP.

the coenzymatic function of niacin is shown in Figure C. In general, NAD-dependent enzymes are involved in catabolic reactions, whereas NADP-dependent systems are more common to biosynthetic reactions (McCormick, 1988).

Nicotinic acid is reported to be a component of glucose tolerance factor (GTF), which facilitates the binding of insulin to its receptor (Mertz et al., 1974). Urberg and Zemel (1987) conducted a study where 16 healthy elderly persons were given 200 μ g chromium (Cr), 100 mg nicotinic acid or both daily for 28 days. Fasting serum glucose and glucose tolerance were not affected by Cr or nicotinic acid alone. The combined Cr-nicotinic acid supplement caused a 15% decrease in a total integrated glucose area and a 7% decrease in fasting serum glucose. The results suggest that the inability to respond to Cr supplementation may result from suboptimal levels of dietary nicotinic acid. Lee et al. (1961) reported that nicotinic acid (but not nicotinamide) had an "insulin-like" effect on rat epididymal fat pad tissue, as it increased the rate of glucose metabolism and lipogenesis. Similarly, Taylor and Halpern (1979) found that nicotinic acid increased the rate of conversion of [1- 14 C]-glucose to 14 CO₂ and the rate of glucose disappearance from the incubation medium in isolated rat adipocytes.

Substrate Oxidized
 Coenzyme

NAD⁺
or NADP⁺

Oxidized Reduced
Substrate Coenzyme

NADH + H⁺
or NADPH + H⁺

Figure C. The mechanism of the coenzymatic function of niacin.

Note. From "Summary of known metabolic functions of nicotinic acid, riboflavin, and vitamin B₆" by E.E. Snell, 1953, Physiological Reviews, 33, p. 510.

Absorption and Metabolism of Niacin

In humans, dietary niacin was shown to be equally well-absorbed from the stomach and upper small intestine by employing the gastrointestinal tube technique (Bechgaard & Jespersen, 1977). Both nicotinic acid and its amide are readily absorbed by diffusion. The metabolism of nicotinic acid is depicted in Figure D. Henderson and Gross (1979) reported that the absorbed nicotinic acid is actively converted to nicotinamide in the intestinal mucosa by the nicotinamide adenine dinucleotide (NAD) pathway (Enzymes a, b, c, and d). The first two steps involve the cytosolic phosphoribosyltransferase-catalyzed reaction (Enzyme a) of nicotinic acid to nicotinic acid mononucleotide which then reacts with ATP to form Deamido-NAD (Enzyme b). In the third step, Deamido-NAD reacts with glutamine and ATP to form NAD (Enzyme c). Finally, NAD is catabolized to nicotinamide by NAD glycohydrolase (Enzyme d). Since direct conversion of nicotinic acid to nicotinamide has not been demonstrated, the formation of nicotinamide in the body takes place only through NAD. Nicotinamide formed in the tissues is transported to the gastrointestinal tract where it can be recycled back to nicotinic acid by Enzyme e (Ijichi et al., 1966). Furthermore, nicotinamide in the tissues can be directly incorporated into NAD by nicotinamide phosphoribosyltransferase (Enzyme f). In

Enzymes:

- a: Nicotinate phosphoribosyltransferase
- b: Mononucleotide adenylyltransferase
- c: Synthetase
- d: NAD glycohydrolase
- e: Nicotinamide deamidase
- f: Nicotinamide phosphoribosyltransferase
- g: Kinase

Figure D. The metabolism of tryptophan and nicotinamide nucleotides.

addition, nicotinamide adenine dinucleotide phosphate (NADP) can be formed by a kinase-catalyzed phosphorylation of NAD (by Enzyme g) (McCormick, 1988).

Tryptophan-Niacin Conversion

In 1945, Krehl and co-workers showed that tryptophan could be substituted for dietary niacin to a certain degree in rats. Assessment of niacin requirements, therefore, takes the contribution of tryptophan into account. In humans, it is estimated that 60 mg tryptophan can be converted to 1 mg niacin (Goldsmith et al., 1961). This conversion ratio varies in different species. Baker et al. (1973) reported a conversion ratio of 45:1 (tryptophan: niacin) in chickens. A conversion ratio of 112:1 in turkeys was obtained by Ruiz and Harms (1990). Ducklings appear to be even less efficient than turkeys with a conversion ratio of 177:1 (Chen & Austic, 1989). The pathway of tryptophan conversion to nicotinic acid mononucleotide in mammals is shown in Figure D.

The utilization of dietary tryptophan, nicotinamide and nicotinic acid as precursors of the nicotinamide nucleotides was studied by Bender et al. (1982). In their studies, rats were fed diets providing only one of these precursors at a time, in amounts adequate to meet their requirements for NAD and NADP synthesis. Enzyme kinetic studies suggested that the three major enzymes involved in the incorporation of

preformed niacin into nucleotides in the liver (Enzymes a, e, and f) (Figure D) acted at or near their maximum rates at normal intracellular concentrations of nicotinamide and nicotinic acid. However, their results suggest that the ability to utilize dietary nicotinamide is very limited, and even when tissue levels of nucleotides are severely depleted, nicotinamide released by hydrolysis cannot be reutilized (Bender et al., 1982). McCreanor and Bender (1986) found that in rats a high dietary intake of tryptophan (5.9 g/kg diet) led to considerable increases in liver NAD/NADP ratio and urinary excretion of the niacin metabolites N¹-methyl nicotinamide and methyl pyridone carboxamide. In rats, administration of a single large oral dose of nicotinamide or nicotinic acid (up to 100 mg/kg body weight) resulted in only a small increase in the liver content of nicotinamide nucleotide coenzymes (both NAD and NADP). The results suggest that the utilization of dietary nicotinamide and nicotinic acid for NAD/NADP synthesis is limited. In contrast, NAD/NADP synthesis from the tryptophan metabolite quinolinic acid is relatively unlimited. Therefore, when preformed niacin is abundant in the tissues, there would be little or no utilization of niacin from the diet or arising from the catabolism of NAD and NADP.

In 1988, Bender and Olufunwa also reported that neither

nicotinic acid nor nicotinamide was utilized to any significant extent as a precursor of NAD and NADP in rat liver, whereas de nova synthesis from tryptophan permitted replacement of these nucleotides. These results indicate that the liver functions synthesis of niacin from tryptophan.

Nutritional Requirements of Niacin in Different Species

In humans, niacin deficiency affects primarily the skin, the gastrointestinal tract, and the central nervous system. Niacin deficiency in humans leads to pellagra. The typical presentation of pellagra is that of a chronic wasting disease associated with "the three D's" deficiency syndrome: dermatitis, diarrhea, and dementia (Wilson, 1982). The Food and Nutrition Board (FNB) has established a Recommended Dietary Allowance (RDA) of 6.6 mg niacin equivalents (N.E.) of niacin per day for adults for every 1000 Kilocalories consumed.

In animal studies, niacin was found to be essential for rabbits, pigs, ducks, minks, chickens, turkeys and other species of animals (Wooley & Sebrell, 1945; Braude & Kon, 1946; Hegsted, 1946; Warner et al., 1968; Powell & Gehle, 1975; Ruiz & Harms, 1988). The common symptoms of niacin deficiency in these animals are decreased growth and diarrhea. The requirements of niacin for different animals are summarized in Table I.

Table I
Niacin Requirements of Different Species

Species	Requirement (mg/kg diet)	Criteria used
Human ¹	26	Optimal intake
Turkey ²	44	Optimal growth
Chicken ³	25	Optimal growth
Duck ⁴	20	Optimal growth
Mink ⁵	10-20	Optimal growth
Rabbit ⁶	10	Optimal growth
Pig ⁷	10	Optimal growth
<u>Entamoeba histolytica</u>	18, ^a	maximum growth
<u>Staphylococcus aureus</u>	0.05 ^{9, a}	maximum growth

¹National Research Council (1989). Based on 6.6 mg/1000 Kcal diet and 2000 Kcal/day/person.

²Ruiz & Harms, 1988.

³Powell & Gehle, 1975.

⁴Hegsted, 1946.

⁵Warner et al., 1968.

⁶Wooley & Sebrell, 1945.

⁷Braude & Kon, 1946.

⁸Weik & Reeves, 1980.

⁹Landy, 1938.

^aμg/ml of medium.

Niacin was also found to be essential for the axenic cultivation of Entamoeba histolytica, a parasitic protozoa (Weik & Reeves, 1980). A culture medium made with liver extract and not supplemented with nicotinic acid failed to support continued multiplication of Entamoeba histolytica, but did support serial subculture when niacin was added. The concentration of niacin required to achieve maximum growth was about 1 µg per ml of medium.

In 1938, Landy reported that in Staphylococcus aureus, 0.05 µg of nicotinamide per ml of medium was sufficient for prompt growth of cultures. In bacteriological studies, nicotinic acid and nicotinamide display different growth-promoting activities (Koser et al., 1941). In Diphtheria bacillus, nicotinic acid showed a distinctly greater growth-promoting effect (the activity of nicotinic acid:nicotinamide is 10:1). The Proteus group of bacteria can apparently make use of nicotinic acid about as effectively as nicotinamide, while the Staphylococcus bacilli and Dysentery bacilli evidently experience more difficulty in the utilization of nicotinic acid (1:5). The Pasteurella group of bacteria is able to utilize nicotinamide, but not nicotinic acid.

Toxicity

Pharmacologic doses of nicotinic acid (but not the amide) are used in humans to lower plasma lipid

concentrations. Therapy is generally begun with single doses of 100 to 250 mg/day. Frequency of dose and total daily dose are gradually increased until a first level therapeutic dose of 1.5 to 2.0 g/day is reached (Rader et al., 1992). The Recommended Dietary Allowances for niacin as a vitamin are 13 to 19 mg/day for adult men and women aged 19 to 51+ years (National Research Council, 1989). Doses of 2 g/day that are therapeutically useful for lowering serum cholesterol levels are approximately 100-fold higher than amounts of the vitamin required to meet normal adult nutritional needs. Flushing is the common side effect of nicotinic acid therapy (Rader et al., 1992). Liver toxicity (e.g. jaundice) is a potentially serious side effect of treatment with nicotinic acid. Rivin (1959) first reported elevated serum transaminase (glutamic oxaloacetic transaminase) levels in a patient treated with nicotinic acid. Nausea, vomiting, anorexia, and weakness have been reported with continued therapy with nicotinic acid (Patterson et al., 1983).

In 1938, Chen et al. reported that with lethal or near-lethal doses of nicotinic acid, mice (4.5 g/kg), rats (3.5 g/kg), and guinea pigs (3.5 g/kg) developed convulsions, and either died promptly or recovered without apparent after effects such as irritability and weight loss. Nicotinamide caused paralysis of the respiratory center when administered

in large doses (1.68 g/kg). In rats nicotinamide was more toxic than nicotinic acid (Brazda & Coulson, 1946). The presence of the carboxyl group on the β position of pyridine decreases the toxicity of the molecule, while conversion of this group to the simple amide markedly increases toxicity. In bacterial studies, the amount of niacin was raised to 1,000 $\mu\text{g/ml}$ before growth was distinctly retarded. In the presence of niacin at 3,000, 5,000 and 10,000 $\mu\text{g/ml}$, growth was progressively retarded and was often completely inhibited at the 10,000 μg level. At 10,000 $\mu\text{g/ml}$ of niacin, the cells appear abnormal on microscopic examination (Koser & Kasai, 1947). The organisms used included some which need preformed nicotinic acid and others which are able to synthesize the vitamin. They include Shigella paradysenteriae, Escherichia coli, Salmonella aertrycke, Salmonella enteritidis, Proteus vulgaris, Proteus morganii, Pseudomonas fluorescens, Serratia marcescens, Agrobacterium tumefaciens, Staphylococcus aureus, Staphylococcus albus, and Bacillus subtilis.

CHAPTER 2
JOURNAL ARTICLE

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THE REQUIREMENT OF NIACIN AND ITS BIOSYNTHESIS FROM
TRYPTOPHAN IN THE NEMATODE, Caenorhabditis elegans¹

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ABSTRACT

The requirement for niacin was determined in the free-living nematode, Caenorhabditis elegans (C. elegans). The growth-promoting activity of 0 (control), 0.060, 0.30, 1.5, 7.5, 38, 190, and 940 $\mu\text{g/ml}$ of nicotinic acid (N), nicotinamide (Nm), and nicotinic acid plus nicotinamide (N + Nm) was quantitatively determined. Based on the population growth of C. elegans, the deficient, optimal, and toxic levels for N or Nm were below 1.5, between 1.5 to 190, and above 190 $\mu\text{g/ml}$, respectively. Nicotinic acid (N) demonstrated greater growth-promoting activity than Nm at all levels, indicating that N is more active than Nm in the nematode. The deficient, optimal, and toxic levels for N + Nm were determined to be below 0.30, between 0.30 to 38, and above 38 $\mu\text{g/ml}$, respectively. In our studies of the role of tryptophan as a niacin precursor, the basal medium contained 0.30 $\mu\text{g/ml}$ of nicotinic acid and 180 $\mu\text{g/ml}$ of tryptophan. The basal medium was supplemented with additional nicotinic acid (0, 0.60, or 1.2 $\mu\text{g/ml}$) or additional tryptophan (0, 180, 370, 550, 740, or 920 $\mu\text{g/ml}$). Using Finney's slope ratio procedure, the conversion ratio of tryptophan to niacin was determined to be 500:1 in C. elegans. Compared with the conversion ratio reported in other animals, our results indicate that tryptophan is relatively ineffective

as a niacin precursor in C. elegans.

INTRODUCTION

Caenorhabditis elegans (C. elegans) is a free-living nematode that has been cultivated under axenic (germ-free) conditions. This nematode has a short life-cycle (34 days), a rapid generation time (3.5 days), and the ability to be cultivated in large quantities for bioassay under defined conditions (Croll and Mathews 1977, Marx 1984). Therefore, it has become widely used as a model for nutritional research (Bolla 1987, Lu et al. 1978, Lu and Goetsch 1993, Zuckerman 1981).

The nutritional requirements of C. elegans for amino acids (Vanfleteren 1973), carbohydrate (Lu and Goetsch 1993), sterol (Hieb and Rothstein 1968, Lu et al. 1977), minerals (Lu et al. 1983), and heme (Hieb et al. 1970) have been investigated. The requirements for several water soluble vitamins (Augustin 1992, Lu et al. 1974, Lu et al. 1976, Sun et al. 1986) have also been determined. In 1962, Nicholas et al. reported that niacin was required by the nematode, C. elegans. However, these authors did not determine the concentration of niacin necessary for optimal growth.

It is well known that the niacin requirements of many animal species can be satisfied by the administration of

tryptophan, and that this amino acid increases intermediate metabolites for the formation of niacin. This was first established for rats by Krehl et al. in 1945. Later, it was found that niacin can be synthesized from tryptophan in other vertebrates (Baker et al. 1973, Chen and Austic 1989, Nishizuka and Hayaishi 1963, Ruiz and Harms 1990) as well as in some microorganisms (Ahmad and Moat 1966, Ortega and Brown 1960, Partridge et al. 1952, Wilson and Henderson 1963). However, the information on the conversion of tryptophan to niacin in C. elegans is not yet available.

The objective of this study was to quantitatively determine the niacin requirement for optimal population growth in the nematode, C. elegans. Two forms of niacin (nicotinic acid and nicotinamide) were tested to study the growth-promoting activity in C. elegans. In addition, the conversion ratio of tryptophan to nicotinic acid and the efficiency of tryptophan as a niacin precursor were investigated.

MATERIALS AND METHODS

Stock Media and Culture. The culture conditions used were adopted from Lu and Goetsch (1993). The C. elegans stock medium was composed of 4% Hi-soy, 1% yeast extract, and 10% heated liver extract (HS-YE-HLE) (Tomlinson and Rothstein 1962). C. elegans was cultivated in 18 x 150 mm

culture tubes containing 5.0 ml of the stock medium. The cultures were incubated at 20°C on a tissue culture rotator at a speed of 1 rpm. The nematodes were harvested by centrifugation, washed in distilled water, and resuspended in 3.0 to 4.0 ml distilled water contained 0.15 ml of Caenorhabditis elegans Maintenance Medium (CeMM) (minus nicotinic acid, nicotinamide, and tryptophan) as an inoculum. The number of nematodes was determined by counting diluted cultures under a dissecting microscope.

Niacin Requirement. The experimental medium used to assess niacin requirements consisted of CeMM (Lu and Goetsch 1993) minus nicotinic acid, nicotinamide, and tryptophan. To determine the amounts of nicotinic acid and nicotinamide required for optimal population growth, the nematodes were grown in experimental media containing a fixed level of tryptophan (180 µg/ml) and varying concentrations of nicotinic acid (0 (control), 0.060, 0.30, 1.5, 7.5, 38, 190, and 940 µg/ml) or nicotinamide (0 (control), 0.060, 0.30, 1.5, 7.5, 38, 190, and 940 µg/ml). In a second experiment, the nematodes were grown in media containing a fixed level of tryptophan (180 µg/ml) and equal concentrations of nicotinic acid and nicotinamide (0 (control), 0.060, 0.30, 1.5, 7.5, 38, 190, and 940 µg/ml each).

C. elegans was grown as described above. Each tube contained 5.0 ml of experimental medium, and each culture

was prepared in quadruplicate. The initial population was the same in each culture (600 nematodes/ml). Population growth was determined weekly, and the final population was counted on day 16.

Tryptophan Requirement. Assessing niacin requirements is complicated by the conversion of dietary tryptophan to niacin in vivo (Krehl et al. 1945, Nishizuka and Hayaishi 1963). In addition, tryptophan is an essential amino acid for *C. elegans* (Vanfleteren 1973). Before determining the conversion ratio of tryptophan to niacin, we determined the level of tryptophan required to support optimal growth of *C. elegans* at an optimal level of niacin. The culture conditions and experimental media were as described above except that varying levels of tryptophan (0 (control), 1.5, 7.4, 37, 180, 370, 740, and 1500 $\mu\text{g/ml}$) and fixed levels of nicotinic acid (7.5 $\mu\text{g/ml}$) and nicotinamide (7.5 $\mu\text{g/ml}$) were used.

Determination of Tryptophan-Niacin Conversion Ratio. To determine the tryptophan-niacin conversion ratio, nematodes were grown in a basal medium containing tryptophan (180 $\mu\text{g/ml}$) and nicotinic acid (0.30 $\mu\text{g/ml}$) but no nicotinamide. Two population growth curves were obtained. For the first growth curve, the media contained varying levels of nicotinic acid (0.30, 0.90, or 1.5 $\mu\text{g/ml}$) and a fixed level of tryptophan (180 $\mu\text{g/ml}$). For the second

population growth curve, the media contained a fixed level of nicotinic acid (0.30 $\mu\text{g/ml}$) and varying concentrations of tryptophan (180, 360, 550, 730, 920, or 1100 $\mu\text{g/ml}$). The initial inoculated population was 580 nematodes/ml. The slope ratio procedure (Finney 1978) was used to determine nicotinic acid-tryptophan relationships by establishing two population growth curves supported by increments of either nicotinic acid or tryptophan. Only the levels of nicotinic acid or tryptophan supported in the linear growth response were used for the multiple regression model. The 95% confidence interval was determined for the conversion ratio by applying Fieller's theorem (Finney 1978).

Statistical Analysis. One-way analysis of variance (ANOVA) with a Sum of Squares Simultaneous Test Procedure (SS-STP) was utilized to determine statistical significance of population growth of C. elegans at various levels of niacin or tryptophan.

RESULTS

Quantitative Requirement of Niacin. The effect of different levels of nicotinic acid (N), nicotinamide (Nm), and the combination of nicotinic acid and nicotinamide (N + Nm) on population growth in C. elegans is shown in Figure 1. We first determined the concentrations of nicotinic acid (N), nicotinamide (Nm), or nicotinic acid plus nicotinamide

(N + Nm) required for optimal growth in C. elegans. When C. elegans was cultivated in media containing no nicotinic acid (N) or nicotinamide (N + Nm), the population of nematodes decreased from the initial inoculum of 600 nematodes/ml to 300 nematodes/ml on day 16. Adding nicotinic acid (N) and/or nicotinamide (Nm) to the media increased population growth. In media containing nicotinic acid (N) alone, growth of C. elegans was optimal over a broad range of concentrations (1.5 to 190 µg/ml). At these levels, there were no significant differences in population growth. However, when nicotinic acid (N) levels were increased to 940 µg/ml or decreased to 0.30 µg/ml, population growth dropped significantly ($p < 0.05$). In experimental media containing nicotinamide (Nm) alone, the population growth response was essentially the same as in media containing nicotinic acid (N) alone. Population growth was relatively constant at nicotinamide (Nm) levels ranging from 1.5 to 190 µg/ml. As nicotinamide (Nm) levels were increased to 940 µg/ml or decreased to 0.30 µg/ml, the nematode population decreased significantly. In media containing both nicotinic acid and nicotinamide (N + Nm), optimal population growth was supported by nicotinic acid plus nicotinamide (N + Nm) at 1.5 µg/ml each. However, population growth was relatively constant over a range of concentrations (0.30 to 38 µg/ml). When nicotinic acid plus nicotinamide (N + Nm)

concentrations were increased to 190 and 940 $\mu\text{g/ml}$ or decreased to 0.060 $\mu\text{g/ml}$, population growth fell significantly.

Growth-Promoting Activities of Nicotinic Acid And/Or Nicotinamide. We also compared the growth promoting effects of the different forms of niacin. Nicotinic acid (N) alone supported higher population growth than nicotinamide (Nm) alone at all concentrations tested (Figure 1). However, the nematodes grew to the highest density in medium supplemented with both nicotinic acid and nicotinamide (N + Nm) at 1.5 $\mu\text{g/ml}$ each. This combination of vitamins also exhibited the greatest toxic effects at 190 and 940 $\mu\text{g/ml}$.

Quantitative Requirement of Tryptophan. The effect of different levels of tryptophan on population growth in C. elegans is shown in Figure 2. Population growth was minimal at tryptophan levels below 37 $\mu\text{g/ml}$ and optimal at tryptophan levels of 180 to 740 $\mu\text{g/ml}$. As the tryptophan level increased to 1500 $\mu\text{g/ml}$, population growth significantly decreased, indicating that this concentration of tryptophan is toxic. The results suggested that the optimal levels of tryptophan required for the growth of C. elegans range between 180 and 740 $\mu\text{g/ml}$. Based on these data, a tryptophan level of 180 $\mu\text{g/ml}$ was used in the experimental medium for determining the conversion ratio of tryptophan to niacin in C. elegans.

Conversion of Tryptophan to Niacin. *C. elegans* was cultivated in media containing varying levels of nicotinic acid (0.30, 0.90, or 1.5 $\mu\text{g/ml}$) and a fixed level of tryptophan (180 $\mu\text{g/ml}$) or in media containing a fixed level of nicotinic acid (0.30 $\mu\text{g/ml}$) and varying levels of tryptophan (180, 360, 550, 730, 920, or 1100 $\mu\text{g/ml}$). Regression analysis of the two population growth response curves for *C. elegans* at 16 days of cultivation on nicotinic acid supplement and tryptophan supplement is shown in Figure 3. Based on the two population growth curves, the amounts of tryptophan required to support the optimal population growth of the nematodes as compared to the optimal population growth supported by the amounts of nicotinic acid can be determined. By using Finney's slope ratio procedure (Finney 1978), an average conversion of 500:1 (tryptophan:niacin) was obtained in this study. The 95% confidence interval for the slope ratio was 350 to 850 (determined by applying Fieller's theorem).

DISCUSSION

Nicholas et al. (1962) reported that the population level of nematodes was relatively reduced when niacin was omitted from basal medium supplemented with a crude tissue extract. The present study demonstrates an absolute

requirement of niacin for population growth in C. elegans. When niacin was omitted from CeMM, the nematode population decreased from the initial inoculation of 600 to 300 nematodes/ml (Figure 1). At niacin (nicotinic acid (N) or nicotinamide (Nm)) levels of 0.060 µg/ml, population growth increased slightly. However, the nematodes appeared much smaller, curled, and lethargic, indicating that growth and reproduction were hindered as a result of niacin deficiency. As levels of nicotinic acid (N) or nicotinamide (Nm) increased to 0.30 µg/ml, population growth increased significantly. However, maximum population growth was not reached until the levels of nicotinic acid (N) or nicotinamide (Nm) increased to 1.5 µg/ml. At 940 µg/ml, the population of C. elegans significantly decreased. Therefore, optimal population growth was achieved at nicotinic acid (N) or nicotinamide (Nm) levels between 1.5 to 190 µg/ml. Deficient and toxic levels of nicotinic acid (N) or nicotinamide (Nm) were determined to be below 1.5 µg/ml and above 190 µg/ml, respectively. Population growth supported by media containing both nicotinic acid and nicotinamide (N + Nm) was slightly greater than growth supported by nicotinic acid (N) or nicotinamide (Nm) alone. Nicotinic acid plus nicotinamide (N + Nm) concentrations between 0.30 to 38 µg/ml each promoted optimal growth of C. elegans. Deficient levels of nicotinic acid plus

nicotinamide (N + Nm) were below 0.30 $\mu\text{g/ml}$ and toxic levels were above 38 $\mu\text{g/ml}$. These results suggested that C. elegans requires a minimum amount of 1.5 $\mu\text{g/ml}$ of nicotinic acid (N) or nicotinamide (Nm) alone or a minimum of 0.30 $\mu\text{g/ml}$ of both nicotinic acid and nicotinamide (N + Nm) in the culture media for optimal population growth.

Compared to nicotinamide (Nm), nicotinic acid (N) supported greater population growth at either deficient or optimal levels, and also had less toxic effect on C. elegans. Our results indicated that nicotinic acid (N) generated approximately 1.5 times greater growth-promoting activity than nicotinamide (Nm) in C. elegans at all levels. In bacterial species, nicotinic acid and nicotinamide also display different growth-promoting activities (Koser et al. 1941). In Diphtheria bacillus, nicotinic acid showed a distinctly greater growth-promoting effect (nicotinic acid:nicotinamide is 10:1). The Proteus group of bacteria can apparently make use of nicotinic acid about as effectively as nicotinamide, while the Staphylococcus bacilli and Dysentery bacilli evidently experience more difficulty in the utilization of nicotinic acid (1:5). The Pasteurella group of bacteria is able to utilize nicotinamide only, and not nicotinic acid. In C. elegans, nicotinic acid plus nicotinamide (N + Nm) supported additional population growth at deficient and optimal levels

due to the levels of nicotinic acid plus nicotinamide (N + Nm) combined being two times greater than the levels of nicotinic acid (N) or nicotinamide (Nm) alone.

Nevertheless, our results showed that the population growth supported by nicotinic acid plus nicotinamide (N + Nm) at 1.5 µg/ml each supported greater population growth than media containing nicotinic acid (N) or nicotinamide (Nm) alone, indicating both forms of niacin are required by C. elegans for optimal population growth.

The metabolic conversion of tryptophan to niacin has been studied extensively in many animals. A ratio of 60:1 (tryptophan:niacin) is accepted for humans (Goldsmith et al. 1961). Baker et al. (1973) determined a conversion ratio of 45:1 in the chicken (Gallus domesticus). A conversion ratio of 112:1 was obtained by Ruiz and Harms in turkeys (1990). Ducklings appear to be even less efficient than turkey poults with a ratio of about 180:1 (Chen and Austic 1989). In this study, we determined that the conversion of tryptophan to niacin was 500:1 in C. elegans. Thus the conversion of tryptophan to niacin in C. elegans appears to be much less efficient than in other animals reported. The growth response of C. elegans to tryptophan (180 to 550 µg/ml) was weak when compared with nicotinic acid at levels of 0.30 to 1.5 µg/ml as described in this study (Figure 3).

The domestic cat appears incapable of synthesizing

niacin from tryptophan (da Silva et al. 1952), a phenomenon which makes it similar to the fly, *Drosophila* (Suchultz and Rudkin 1948), and other insects (Fraenkel and Stern 1951). The nicotinic acid requirements of two insect species, *Tribolium confusum* and *Tenebrio molitor*, have been studied in relation to the tryptophan content of their diets. It was found that dietary tryptophan could not compensate for the lack of nicotinic acid.

A tryptophan-niacin pathway (Figure 4) has been described in mammalian liver (Nishizuka and Hayaishi 1963), *Neurospora* (Partridge et al. 1952) and aerobically grown yeast (Ahmad and Moat 1966). Most bacteria (Ortega and Brown 1960) do not appear to utilize tryptophan for niacin biosynthesis, except for *Xanthomonas pruni* (Wilson and Henderson 1963). Yanofsky (1954) reported that a series of nicotinic acid auxotrophs of *Escherichia coli* and *Bacillus subtilis* could utilize neither tryptophan nor any of the intermediates in the tryptophan pathway as a replacement for nicotinic acid as a growth factor. Also, these bacteria appeared to lack the enzyme, kynureninase, which splits 3-hydroxykynurenine to 3-hydroxyanthranilic acid (see Figure 4) and which is essential for the conversion of tryptophan to nicotinic acid.

Nutrient metabolism in *C. elegans* is similar to that in humans and rats. In 1974, Lu et al. found that accumulation

of formimino-L-glutamic acid (FIGLU) in nematode tissues is related to folic acid deficiency. A low lactate to pyruvate ratio is an indicator of thiamin deficiency in C. elegans (Augustin 1992). During the tryptophan-load test, tryptophan metabolites (xanthurenic and kynurenic acids) accumulate in the culture medium of vitamin B₆ deficient nematodes (Sun et al. 1986). However, we show in the present study that the conversion of tryptophan to niacin is very low in C. elegans. Sun et al. (1986) reported that nematodes are able to metabolize tryptophan to 3-hydroxyanthranilic acid. Our results suggest that nicotinic acid can be converted to NAD through nicotinic acid mononucleotide. Therefore, the metabolic defect in the conversion of tryptophan to NAD appears to occur between 3-hydroxyanthranilic acid and nicotinic acid mononucleotide in C. elegans (Figure 4).

In summary, we demonstrate optimal growth of C. elegans at nicotinic acid plus nicotinamide concentrations of 0.30 to 38 µg/ml each. However, this species is unable to convert tryptophan to niacin effectively.

Figure 1 Effect of different levels of nicotinic acid (N), nicotinamide (Nm), and nicotinic acid plus nicotinamide (N + Nm) on population growth in *C. elegans*. Each point is the mean of quadruplicate; values represent mean $\pm$ SD. Values at each line sharing a common letter are not significantly different ($p > 0.05$). Statistical comparisons were made with Sum of Squares Simultaneous Test Procedure (SS-STP). Tryptophan was fixed at 180 $\mu\text{g/ml}$. Glucose (32.5 mg/ml) was added to the medium as an energy source. The concentration of N + Nm is the sum of N and Nm for each level.

Figure 2 Effect of different levels of tryptophan on population growth in *C. elegans*. Each point is the mean of quadruplicate; values represent mean $\pm$ SD. Values at the line sharing a common letter are not significantly different ($p > 0.05$). Statistical comparisons were made with Sum of Squares Simultaneous Test Procedure (SS-STP). Concentrations of nicotinic acid (7.5 $\mu\text{g/ml}$) and nicotinamide (7.5 $\mu\text{g/ml}$) were added to the medium. Glucose (1.3 mg/ml) and potassium acetate (5.0 mg/ml) were added to the medium as energy sources.

Figure 3 Regression of the population growth for *C. elegans* at 16 days of cultivation (Y) on nicotinic acid supplement (X_1) or tryptophan supplement (X_2). The open circles represent the growth responses from the nicotinic acid supplement, and the open squares represent the growth responses from the tryptophan supplement for *C. elegans*. The two lines, one light and one dark, represent the predicted equations for nicotinic acid and tryptophan respectively. $Y = (47.6 + 15.56X_1 + 0.0312X_2) \times 10^3$. The Initial population was 580 nematodes/ml. Glucose (32.5 mg/ml) was added to the medium as an energy source.

Figure 4 The metabolism of tryptophan and nicotinamide nucleotides.

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CHAPTER 3

SUMMARY AND RECOMMENDATIONS

Summary

The requirement of niacin (nicotinic acid, nicotinamide, and nicotinic acid plus nicotinamide) in C. elegans was investigated. Growth-promoting activity of nicotinic acid and nicotinamide were quantitatively determined. Conversion of tryptophan into nicotinic acid was measured using Finney's slope method. The results showed that the deficient, optimal, and toxic levels for nicotinic acid or nicotinamide were below 1.5, between 1.5 to 190, and above 190 $\mu\text{g/ml}$, respectively. The deficient, optimal, and toxic levels of nicotinic acid plus nicotinamide were below 0.30, between 0.30 to 38, and above 38 $\mu\text{g/ml}$, respectively. Nicotinic acid alone in the experimental medium showed greater growth-promoting activity than Nm alone at either deficient, optimal, or toxic levels. Using Finney's slope ratio procedure, a conversion ratio of 500:1 (tryptophan:niacin) was obtained for C. elegans. Our results indicate that tryptophan is less effective as a niacin precursor in C. elegans than reported in other animal species.

RECOMMENDATIONS

To improve the experimental design of this study, the following recommendations are made.

1. In the tryptophan to niacin conversion experiment, eight culture tubes should be cultivated per level of tryptophan and niacin instead of four tubes to increase the sample size for statistical analysis.

2. The increment level of tryptophan (180 $\mu\text{g/ml}$) in the conversion experiment should be decreased to 50 to 100 $\mu\text{g/ml}$ in order to determine the peak population growth for the tryptophan supplementation.

3. To determine the major limiting step in the conversion of tryptophan to niacin in C. elegans, the growth-promoting effects of the intermediate metabolites (kynurenine, 3-hydroxykynurenine, 3-hydroxyanthranilic acid, and quinolinic acid) should be tested.

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Appendix I

COMPONENTS OF CAENORHABITIS ELEGANS MAINTENANCE MEDIUM^a

I.	Vitamins & Growth Factors	MW ^b	gm ^c
	N-Acetylglucosamine	222.2	0.01500
	Cyanocobalamine	1355.4	0.00375
	Niacinamide	122.1	0.00750
	Pantethine	554.7	0.00375
	Pantothenate (Ca)	238.3	0.00750
	Pyridoxamine.2HCL	241.1	0.00375
	Pyridoxine.HCL	205.6	0.00750
	Pyridoxal.PO ₄	247.1	0.00375
	Riboflavin-5'-PO ₄ (Na) .2H ₂ O	514.4	0.00750
	Thiamin.HCL	337.3	0.00750
	Biotin	244.3	0.00375
	Niacin	123.1	0.00750
	Pterolylglutamic Acid	441.4	0.00750
	DL-Thioctic Acid	206.3	0.00375
	Gluthione, reduced	307.3	0.20400
	Choline H ₂ Citrate	295.3	0.08850
	myo-Inositol	180.2	0.06450
	p-Aminobenzoic	137.1	0.00750
II.	Salts		
	CaCl ₂ .2H ₂ O	147.0	0.2205
	CuCl ₂ .H ₂ O	170.5	0.0065
	MnCl ₂ .4H ₂ O	197.9	0.0222
	ZnCl ₂	136.3	0.0102
	KH ₂ PO ₄	136.1	1.2255
	K ₃ Citrate.H ₂ O	324.4	0.4860
	Fe (NH ₄) ₂ (SO ₄) ₂ .6H ₂ O	392.2	0.0588
	Mg (OH) ₂	58.3	0.1740
	Citric Acid.H ₂ O	210.1	0.6303
III.	Amino Acids		
A.	Essential Amind Acids		
	L-Arginine	174.2	0.9750
	L-Histidine	155.2	0.2830
	L-Lysine.HCL	182.6	1.2830
	L-Tryptophan	204.2	0.1840
	L-Methionine	149.2	0.3890
	L-Threonine	119.1	0.7170
	L-Leucine	131.2	1.4390
	L-Isoleucine	131.2	0.8610
	L-Valine	117.1	1.0200
	L-Phenylalanine	165.2	0.6230

Appendix I (continued)

B. Non-essential Amino Acids	MW ^b	gm ^c
L-Phenylalanine	165.2	0.1800
L-Tyrosine	181.2	0.2720
L-Alanine	89.1	1.3950
L-Aspartic Acid	133.1	1.6200
L-Cysteine.HCL.H ₂ O	175.6	0.0280
L-Glutamate (Na).H ₂ O	187.1	0.5500
L-Glutamine	146.2	1.4630
Glycine	75.1	0.7220
L-Proline	115.1	0.6530
L-Serine	105.1	0.7880
IV. Nucleic Acid Substituents		
Adenosine-3'-(2')-Phosphoric Acid.H ₂ O	365.2	0.3652
Cytidine-3'-(2')-Phosphoric Acid	323.2	0.3232
Guanosine-3'-(2')-PO ₄ (Na) ₂ .H ₂ O	425.2	0.3632
Uridine-3'-(2')-Phosphoric Acid	324.2	0.3242
Thymine	126.1	0.1261
V. Other Growth Factors		
Cytochrome C	12384.0	0.0500
β-Sitosterol	414.7	0.0500
VI. Energy Source		
D-glucose	180.2	32.5000
or K-Acetate ^d	98.1	5.0000
VII. Solvents		
KOH	56.1	e
Triethanolamine (TEA)	149.2	0.0325
Tween 80	1308.0	1.2500

^aLu and Goetsch, 1993.^bMolecular Weight^cgm/500 ml (2X)^dA minimal 1.3 gm/500 ml glucose is also included^eNeeded for adjustment of pH to 5.9 ± 0.1