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## Role of nicotinic acid in nutrition

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THE ROLE OF NICOTINIC ACID IN NUTRITION

by

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## INTRODUCTION

Casimir Funk's epoch-making discovery in 1911 that beri-beri was probably caused by a deficiency in the diet of very small amounts of a substance which he called a "vitamine" (because he considered it essential to life and thought it to be in the nature of an amine) started off a field of research which has probably never been equaled in scientific history. Since Funk's original discovery tremendous strides forward have been made in our knowledge of these factors called vitamins in a remarkably short time. However, there still remains much to be learned about the action of these extraordinary substances in the body before we can really consider our knowledge adequate, and before we can successfully combat much of the present-day faddism associated with vitamin therapy.

The purpose of this paper is to review the development of our present knowledge of one of these vitamins, nicotinic acid, and to evaluate, insofar as is possible, its efficacy in the treatment of certain clinical conditions. Although every medical practitioner recognizes the value of nicotinic acid in the treatment of pellagra, there are probably many who do not realize its importance in the less evident manifestations of deficiency and certainly many who do not know of its significance in body physiology and chemistry. Much must be left unwritten at the present time, for our knowledge of this substance is still in its infancy, but investigative attention on this substance is continuing and increasing so rapidly

that we may hope for full clarification of the subject before many years have passed.

Since I have been working with Dr. Sergius Morgulis, Professor of Biochemistry at the University of Nebraska College of Medicine, on the determination of nicotinic acid in the blood and urine, I had hoped to be able to contribute some original information in this paper. However, our work has not yet advanced to the stage where anything of clinical significance can be contributed, but I have included some suggestions for improvements in the chemical assay of nicotinic acid.

## HISTORICAL REVIEW

Nicotinic acid was first prepared in 1867 by Huber in his study of nicotine. He obtained a compound which he gave the formula  $C_6H_5NO_2$  by oxidizing nicotine with acid potassium chromate and sulfuric acid. He characterized this substance correctly as an acid with a pyridine base ( $C_5H_5N$ ). (1) The presence of nicotinic acid in biological material was not suspected, however, until Suzuki, Shimamura, and Odake isolated it from rice-polishings in the course of their investigations on beri-beri and polyneuritis. (2) Funk independently isolated nicotinic acid from both yeast and rice-polishings in 1913 in his attempt to isolate the "vitamine" which would prevent beri-beri. (3) Unfortunately, since investigative attention was at that time being focused on beri-beri, the real significance of this discovery was not realized, and further work with nicotinic acid was abandoned when Funk found it to have no effect in the cure of beri-beri or polyneuritis. (3) It is of interest to note, however, that Funk made the prophetic statement that there probably existed many undiscovered vitamins, other than the anti-beri-beri vitamin, which might one day be effective in the prevention and cure of rickets, scurvy, and pellagra. (4)

Since nicotinic acid has proved to cure pellagra so dramatically, and since investigation of the etiology and treatment of pellagra is so intimately connected with the story of nicotinic acid, it will be of interest to recount what is known of the history of

pellagra.

As far as is known no accurate description of pellagra as a specific disease entity was presented before 1735. In fact, the disease was probably confused with scurvy, syphilis, and leprosy. In 1735 Don Gaspar Casal, physician to Philip V of Spain, wrote a description of a serious disease entity which he had recognized among the peasants of Asturia (a province in Spain). The Spaniards called this condition mal de la rosa (rose sickness). At first Casal mistook the disease for an atypical form of leprosy because of its severe, ravaging effects, and he called it "scorbutic leprosy". He so accurately described the skin lesions and the digestive and mental disturbances that there is no doubt that he was dealing with severe forms of what we now call pellagra.

The next report of the existence of this disease in any serious proportions came from Italy in 1771. At this time the disease was reported by the Italian physician, Frapolli, who gave it the name we now use, pellagra. The term was derived from the Italian, pellis aspera (rough skin), and had been in common use among the peasants of Lombardy as their name for this disease. Some toxin from spoiled maize was incriminated as the cause of pellagra by Lombroso in Italy at about this time. This theory gained such wide credence that in 1776, due to the serious proportions which pellagra had assumed, legislation was enacted in Italy to control the sale of maize.

The first case of pellagra in the United States to be reported

was that of Gray in 1864. Tyler also reported a case in 1864. Sporadic reports of pellagra were published after this, but the seriousness of the condition was not realized until 1906 when Dr. George Searcy, then in charge of the Alabama State Hospital for Insane Negroes at Mount Vernon, recognized that an epidemic of a new and fatal disease which had been occurring among the inmates for a year past was, in reality, pellagra. Following this report there were other reports of outbreaks in various state hospitals for the insane, especially in the South, and within a few years cases had been reported from almost every state in the Union.

Within the next few years many state pellagra commissions were formed, and in 1912 a National Pellagra Conference was called. In 1913 the United States Public Health Service turned its attention to the disease and appointed Joseph Goldberger as head of a group to investigate the etiology and the possibilities of cure and prevention of pellagra. Although food deficiency had been suspected as the cause of pellagra for over a hundred years, and although improvement of dietary intake, especially as regards meat and milk, had been the main method of treatment of pellagra as far back as the time of Casal's original work, it remained for Goldberger to actually prove the theory of dietary deficiency as the cause of pellagra. (4)

As a result of experiments on institutional patients with pellagra who were cured by dietary change alone, and on the experimental production of pellagra in convicts by dietary restriction,



Goldberger postulated his theory in 1916 that an "unbalanced diet, low in proteins" was the cause of pellagra. He suggested at this time that "the inclusion of fresh animal and leguminous protein foods in the diet" would prevent pellagra. (5)

Further evidence supporting dietary deficiency as the cause of pellagra began to be accumulated from animal experimentation. In 1916 Spencer called attention to the fact that a condition called black tongue, occurring spontaneously in dogs, exhibited marked similarities to pellagra in humans. (6) In 1917 Chittenden and Underhill reported the results of experimental work on dogs begun as early as 1905 in which they had produced a syndrome which was similar to human pellagra by feeding restricted diets of cooked peas, crackermeal, and cottonseed oil. They expressed the opinion that the condition was a deficiency disease, and was probably not caused by a defective nitrogen balance, but by some dietary factor such as a vitamin. (7)

In 1920 Cary pointed out the close resemblance between the Chittenden-Underhill syndrome and canine black tongue. He classed black tongue among the deficiency diseases and again stressed the similarity of the condition to human pellagra. (8) Wheeler, Goldberger, and Blackstock began their work on the experimental production of black tongue in dogs in 1922. They produced the condition by feeding restricted diets similar to those which had been effective in causing experimental pellagra in prison volunteers, thus furnishing further evidence for the common etiology of canine

black tongue and human pellagra. (9) It is interesting to note in this connection that Underhill and Mendel concluded in 1928 that the Chittenden-Underhill syndrome in dogs was not the same as the experimental black tongue produced by Wheeler and Goldberger. They found, in fact, that their disease was cured by butter fat, carotene, and carrots, and concluded that the dietary factor lacking in their syndrome was related to the carotenoids, whereas butter fat had failed to cure Goldberger's black tongue syndrome. (10)

It was not until 1925 that Goldberger finally abandoned his theory of protein deficiency as the cause of pellagra and expressed the concept of a "pellagra-preventive factor" which could be considered the sole factor necessary for the prevention of this disease. He found, from experiments on pellagrins, that brewer's yeast, milk, and lean beef had an especially high content of this factor. (11)

Further isolation and identification of this "pellagra-preventive factor" was made possible through the discovery that it was a part of the vitamin B complex, separate and distinct from the anti-neuritic vitamin. In February, 1926, Smith and Hendrick found that there was some factor in yeast other than the anti-neuritic vitamin which was essential in nutrition. This factor withstood autoclaving at 15 pounds pressure for six hours and was shown not to be a part of the yeast protein. (12) Later in the same month Goldberger et al reported results of experiments on dogs in which yeast and lean beef were effective in the cure of black tongue even after

autoclaving, whereas these were not effective in the cure of polyneuritis after they had been autoclaved. Goldberger suggested that his "factor P-P" was probably related to vitamin B (the anti-neuritic vitamin) but was not destroyed by autoclaving as was the anti-neuritic fraction. He called the entire complex "water-soluble vitamin B". (13)

The first hint that nicotinic acid might play an important part in the body economy came from the work of Warburg and Christian on coenzyme in 1935, when they suggested that nicotinic acid amide was a part of the coenzyme molecule. (52) Further work by Warburg and Christian and by von Euler and his associates established the importance of nicotinic acid in biological oxidations, as a part of both coenzyme I and coenzyme II. No connection between these substances and the general subject of pellagra-prevention was yet realized, however.

In 1935 and 1936 Koehn and Elvehjem made a further important step toward the identification of the black tongue-preventive factor when they showed that the flavins would not cure "chick pellagra" or canine black tongue and that liver concentrates from which the flavins had been removed by adsorption on fuller's earth were still effective in curing or preventing both experimental conditions. They thus proved that the portion of the B-complex then called B<sub>2</sub> or G (the heat-stable portion) contained at least two factors, a flavin and the pellagra-preventive (or black tongue-preventive) factor. (14a, 14b)

In the early part of 1937 Funk and Funk reported that rats given nicotinic acid and especially nicotinic acid amine along with their diet had a larger food intake and showed better weight gain than controls on similar diets without nicotinic acid or its amide. (15) They apparently did not connect this with any disease-preventing power. In fact, it is now known that rats are able to synthesize nicotinic acid in the body and, therefore, are not subject to the production of a pellagra-like syndrome experimentally. (64)

It was later in 1937 that Elvehjem and his co-workers published the preliminary report of their now-famous work on the final isolation and identification of the black tongue-preventive factor. (16) They found that nicotinic acid or nicotinamide, when given to dogs on a modified Goldberger diet, would cure black tongue very rapidly and would maintain growth in these dogs although they continued on the same deficient diet. Nicotinamide was isolated from liver, and it was found that 50 milligrams of the nicotinamide had a curative effect on black tongue equal to that of 200 grams of fresh liver. (16,17)

The probable identity of nicotinic acid with the pellagra-preventive factor suggested by Elvehjem's discovery was appreciated almost immediately by the medical profession, and several groups of clinicians who were especially interested in pellagra tested the effect of nicotinic acid on pellagrins within a few months after Elvehjem's original report. All of these groups reported almost miraculous cures of human pellagra. Among the first to report successful cases of pellagra with nicotinic acid were Fouts,

Helmer, Lepkovsky, and Jukes (18), Smith, Ruffin, and Smith (19), and Spies, Cooper, and Blankenhorn.(20) The reports of the work of these groups were followed by a rapid succession of reports of innumerable cures of pellagra with nicotinic acid, its sodium salt, or nicotinic acid amide.

Further work has been done since on the possible uses of nicotinic acid in other conditions, its toxicity, its place in the body economy, the detection of deficiency of nicotinic acid, and various other aspects. These will be taken up in detail later in the body of this paper.

## CHEMICAL DESCRIPTION

Nicotinic acid is the simplest of the known vitamins as regards its chemical structure. It is also known as pyridine-beta-carboxylic acid and 3-pyridinecarboxylic acid. Recently, a change in terminology has been suggested by a committee composed of Drs. Elvehjem, Sebrell, and Spies. This committee was appointed by the Food and Nutrition Board of the National Research Council to adopt a name for nicotinic acid which would not suggest its relationship to nicotine and would, thus, be more suitable for commercial use. These men submitted the terms niacin and niacin amide as alternates for nicotinic acid and nicotinic acid amide. The suggested terms were accepted for use on commercial products, but the original terms of nicotinic acid and nicotinamide are to be retained as first choice for use in scientific literature. (21)

Nicotinic acid is a mono-carboxylic acid derivative of pyridine. Its empirical formula is  $C_5H_4NCOOH$ . Its structural formula and those of its sodium salt and of its amide are represented graphically in figure 1. In the pure state nicotinic acid occurs as colorless, needle-like crystals or as a white crystalline powder with a slightly bitter taste. Its molecular weight is 123.11. It melts between  $234^{\circ}$  and  $237^{\circ}$  C. and is sublimable. It is stable in air and is not hygroscopic. It is slightly soluble in cold water or alcohol and is freely soluble in hot water, hot alcohol, or warm glycerine. It is also soluble in solutions of alkali

carbonates, alkali hydroxides, and dilute mineral acids, but it is practically insoluble in ether. A saturated solution of nicotinic acid has a pH of about 4.7. Nicotinic acid is stable at high temperatures and may be sterilized by autoclaving without decomposition.

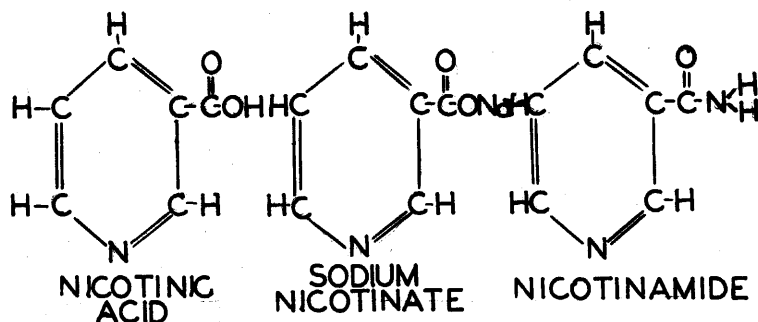


Fig. 1.--Structural Formulas of Nicotinic Acid and Related Compounds

Nicotinic acid amide or nicotinamide has the empirical formula  $C_5H_4NCONH_2$  and a molecular weight of 122.12. The accepted chemical name for this substance is 3-pyridinecarboxamide. It occurs as fluffy, needle-like, white, odorless crystals with a slightly bitter taste. The melting point is between  $129^\circ$  and  $131^\circ C$ . It is very soluble in water and in alcohol, but is only slightly soluble in acetone, ether, benzene, and glycerine. A 2% aqueous solution of nicotinamide has a pH of 5.9. Nicotinamide is stable at temperatures below  $50^\circ C$ , but it may deteriorate on exposure to temperatures above  $50^\circ C$  for long periods of time. Prolonged exposure to light may also cause decomposition.

Since nicotinic acid is not very suitable for parenteral administration its sodium salt is used for this purpose. This substance also occurs as fluffy, white, needle-like crystals with a

slightly bitter taste. Solutions of sodium nicotinate are best for intravenous administration because of the great solubility of the substance and because of the slightly alkaline reaction of its solutions. A 1% solution of sodium nicotinate has a pH of 7.4, a 10% solution a pH of 7.8, and a 20% solution a pH of 8.1. (22,23,24)



## METHODS OF ASSAY

In 1899 Vongerichten first described a reaction of certain pyridine derivatives with 2,4-dinitrochlorobenzene to produce a substance which, on decomposition by amines or alkali hydroxides, yielded a deeply colored product. (25) This reaction was applied to the determination of nicotinic acid and nicotinamide by Karrer and Keller ~~in~~ in 1938 after the importance of these substances in nutrition was made apparent. They fused 2,4-dinitrochlorobenzene with the unknown at 90°C for one hour. Then the fused product was extracted with ether and water, and the watery extract, which contained the nicotinic acid and amide, was made alkaline with KOH. The resulting colored product was then observed in a photoelectric colorimeter, and the amount of nicotinic acid or nicotinamide was calculated by means of extinction curves on the colorimeter. (26)

Vilter, Spiess, and Mathews applied the same method to the determination of nicotinic acid or its amide at about the same time independently. Almost the only difference between their method and that of Karrer and Keller was the use of NaOH in place of KOH. They applied the method especially to the determination of pyridine derivatives in human urine. They found that nicotinic acid and its amide, and possibly other pyridine-like substances, could be determined by this method, but that trigonelline, the chief elimination product of nicotinic acid, could not. (27)

In 1904 König described a somewhat similar reaction to that

of Vongerichten. He showed that upon reaction of various pyridine compounds with cyanogen bromide, addition compounds were formed which, after decomposition with aromatic amines, yielded colored products whose color intensity was proportional to the amount of pyridine substance used. (28) Swaminathan applied this reaction to the determination of nicotinic acid in biological materials. He first decolorized an aqueous extract of the substance to be tested with lead acetate and charcoal, then added cyanogen bromide and aniline. The intensity of the resultant yellow to yellow-green color was then determined by means of a photoelectric colorimeter and the amount of nicotinic acid calculated from previous standardization of the instrument with known quantities of nicotinic acid. In determinations on blood Swaminathan first precipitated the blood proteins by the generally-used Folin tungstic acid method. Swaminathan also showed that trigonelline produced no color in this procedure. (29)

Melnick and Field studied the same method for determining nicotinic acid. After studying various methods of preliminary purification and decolorization of the solutions to be tested they recommended the use of preferential charcoal adsorption to decolorize any colored solution before adding the cyanogen bromide and aniline as the best procedure. (30)

In 1939 Bandier and Hald described a slightly different method for the estimation of nicotinic acid. Colored materials were first precipitated by acetone (in which nicotinic acid is soluble), and

then the acetone-soluble portion was treated with cyanogen bromide and metol (para-methylaminophenol sulfate) with a resulting colored product whose intensity could be determined colorimetrically. These authors stated that nicotinic acid amide had to be hydrolyzed with NaOH to nicotinic acid before it could be determined by their method. (31) A rather similar method, using para-aminoacetophenone instead of aniline or metol to develop the color was reported by Harris and Raymond in 1939. They used this method only on the urine. (32)

The method which Dr. Sergius Morgulis and I have been using in the determination of nicotinic acid and associated compounds is a modification of the cyanogen bromide-aniline method which had been worked out by Friedemann, Barborika, and Haugen within the past year. (33) We consider this the best of the methods reported to date, for it is not only relatively simple, but it gives very consistent results if carried out carefully. Furthermore, it determines both nicotinic acid itself and all other pyridine compounds related to nicotinic acid except trigonelline. These various compounds are hydrolyzed either to nicotinic acid or to some similar compound which gives the same color reaction by means of the HCl used in this procedure. Trigonelline is not hydrolyzed by acids.

Briefly the procedure is carried out in the following manner. To 5 cc. of the blood or urine or to 5 grams of tissue are added 5 cc. of water and then 5 cc. of 8 N HCl in a Kjeldahl digestion tube which is marked at 35 cc. The contents are mixed and the tube

is then covered by a glass bulb and placed in a vigorously-boiling water bath. The digestion is continued for thirty minutes if urine is being analyzed, sixty minutes in the case of blood or tissue. The contents are mixed at frequent intervals during the boiling. After the required time the tube is removed and cooled. The contents are then diluted to about 20 cc. with water, and 2 cc. of zinc sulfate are added. Then 5 cc. of a specially prepared 4 N NaOH solution are added with constant mixing. The tube is again cooled, and then another 5 cc. of the NaOH are added, slowly and with constant mixing with a long, thin, footed, glass rod. The tube is again cooled, and a drop of caprylic alcohol is added, after which the contents are diluted to 35 cc. with water and then mixed thoroughly. The material is then centrifuged at high speed for about 10 minutes. The supernatant fluid should now be perfectly clear and should be just alkaline to phenol red but still colorless to phenolphthalein. This procedure has now precipitated the proteins, has adsorbed practically all color (by means of the zinc hydroxide precipitate), and has hydrolyzed all the pyridine compounds except trigonelline to nicotinic acid or a nicotinic acid-like compound.

Two 5 cc. aliquotes are now measured from the supernatant fluid obtained in the above procedure. To each of these in a colorimeter tube is added 4 cc. of a  $\frac{1}{2}$  M cyanogen bromide solution and 1 cc. of a 4% solution of aniline in absolute alcohol. The intensity of the resultant color is then determined by means of

the Evelyn photoelectric colorimeter using the 420 filter. (33)

Dr. Morgulis and I have found that the color is fully developed within two minutes after adding the aniline and that it remains stable for at least one or two minutes thereafter. We have, therefore, taken our readings on the colorimeter at either two or three minutes after adding the aniline. We have also found that there is less likelihood of turbidity or other disturbing factors in the solution if the material is filtered just after centrifuging, before measuring the 5 cc. aliquotes for the actual determination.

The amount of nicotinic acid present is calculated by means of a curve previously prepared by using known amounts of nicotinic acid in the above procedure to standardize the colorimeter. This standardization was done by using five standard solutions of nicotinic acid which were so prepared that the 5 cc. aliquote of each solution, after the zinc hydroxide precipitation was carried out as described above, contained, respectively, 2.5, 5.0, 7.5, 10.0, and 15.0 micrograms of nicotinic acid. A blank procedure was also carried out using distilled water in place of the unknown to be analyzed. By means of the blank the colorimeter was set at 100, which signified the point of least absorption of light for the procedure, and thus the reading for no nicotinic acid. Readings were then made on the standard solutions after following the procedure previously described, and these readings were plotted on semi-logarithmic graph paper against the known amounts of nicotinic acid used. The resultant curve was a straight line. (Fig. 2)

This curve was then used to read the amount of nicotinic acid in a sample from the amount of light absorbed after development of the color with cyanogen bromide and aniline. This value was then multiplied by 140 to convert to micrograms per 100 cc.

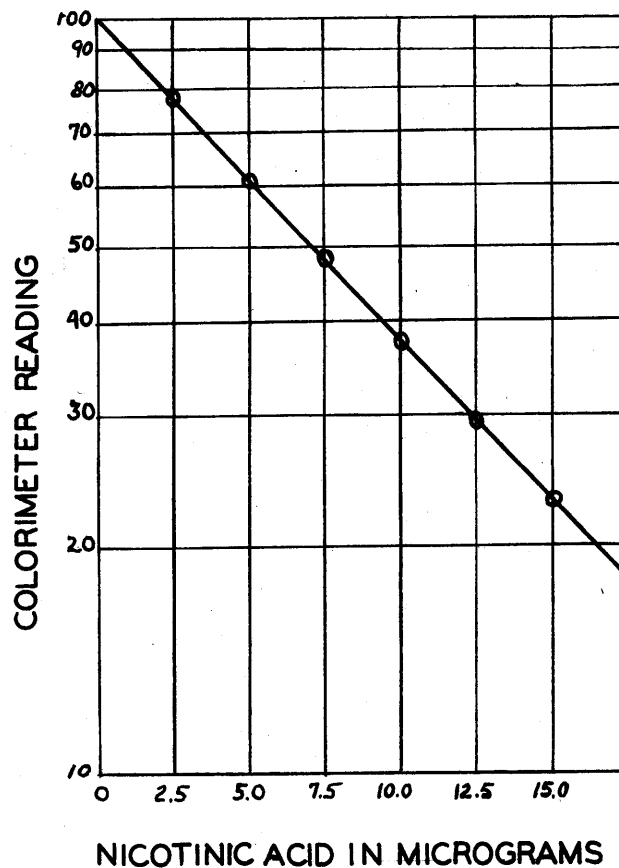


Fig. 2.—Standardization Curve for Nicotinic Acid on Evelyn Photoelectric Colorimeter

I have described this procedure in detail purposely, for I think that it is not only the simplest, but probably the most accurate of the various chemical methods for determining nicotinic acid in biological material. If blood or urine levels of nicotinic

acid should prove to be of clinical value in diagnosis of deficiency states, I am sure this method would be easily adaptable for clinical use. However, blood and urine nicotinic acid levels have not yet been proved to bear any significant relation to nutritional state as I shall bring out in more detail in the following chapter.

I have not yet described a method for determining trigonelline in the urine. This is obviously of great importance, as trigonelline is the chief elimination product of nicotinic acid. In 1940 Perlzweig and his associates suggested a simple method for determining this substance. They found that if trigonelline was heated with 6 N KOH at 75°C in the presence of an ammonium salt or urea it could be determined by the ordinary cyanogen bromide-metol method. These workers obtained a 70% yield by this method. (34) The same procedure can probably be used with the cyanogen bromide-aniline method or the 2,4-dinitrochlorobenzene method if desired.

There are two methods of indirectly estimating the amount of nicotinic acid in biological materials which are fairly widely used. These both depend on the fact that nicotinic acid is found in the body chiefly as a component of the coenzymes I and II. These methods measure the amount of coenzyme present and thus, indirectly, the amount of nicotinic acid.

One of these methods is that used so extensively by Axelrod and Elvehjem in their studies on animal tissues and blood. This is the so-called Euler-Myrbäck yeast fermentation method. The essential procedure is based on the fact that the addition of varying

amounts of coenzyme I to special washed yeast preparations will produce rates of fermentation which are proportional, within certain limits, to the amount of coenzyme added. The coenzyme is extracted by placing the tissue in boiling water for two minutes. The material is centrifuged and 0.4 to 0.8 cc. of the supernatant fluid is used in the determination. This fluid is added to a mixture of a washed yeast preparation (which, thus, contains all enzymes except coenzyme I) with a glucose solution, a solution of hexosediphosphate, a magnesium and manganese solution, and a buffer solution. The rate of carbon dioxide evolution is measured in a Barcroft differential respirometer. A standardization curve for the procedure is prepared by using known amounts of coenzyme I in the same procedure. The unknown may then be calculated from this curve. (35)

The other indirect method for determining nicotinic acid through coenzyme estimation depends on the discovery in 1937 by Iwoff and Iwoff that a certain factor, which they called factor V, was necessary for the proper growth of *Hemophilus influenzae* and *Hemophilus parainfluenzae*. They found that coenzymes I and II were the only known substances which could be shown to have this growth-promoting effect on the hemophile group of bacteria. No other pyridine derivatives could be substituted. (36)

In 1938 Kohn used this discovery as the basis for a method of estimating the amount of coenzyme in blood. The essentials of the method are that the growth-promoting activity of unknown materials



is compared to that of known amounts of coenzyme on cultures of *Hemophilus parainfluenzae*. The cultures are compared for the amount of light absorption (and, thus, indirectly for the number of bacteria) in a photoelectric colorimeter after about 24 to 30 hours of incubation at 37°C. (37)

Although biological assay of unknown material by observing the response of definitely deficient animals to whom this material has been fed is possible with nicotinic acid as with all the other vitamins, this method has not been used very extensively because of the simpler chemical methods of assay. Elvehjem and his associates have applied this method, using black tongue dogs, to check the accuracy of chemical methods. (40)

## OCCURRENCE

In Foods

Although the fact that nicotinic acid is the pellagra-preventive factor has only been established since 1937, the value of certain foods in preventing and curing pellagra has been known for many, many years. Goldberger found, as early as 1925, that brewer's yeast, milk, and lean beef were high in content of his "factor P-P".(11)

Actual estimation of nicotinic acid content of foods by chemical or biological assay was not begun until about 1939, more than a year after the discovery that nicotinic acid was the pellagra-preventive factor. The first report of the chemical estimation of nicotinic acid in foodstuffs was that of Bandier in July, 1939. He used the cyanogen bromide-metal method of estimating nicotinic acid and found the following amounts of nicotinic acid per 100 grams of fresh material: 5 mgm. in pork and beef muscle, 12 mgm. in pork and beef liver, about 15 mgm. in several commercial stomach preparations, about 50 mgm. in several yeast preparations, and from 30 to 120 mgm. in several commercial liver preparations and extracts. (38)

In 1940 Kodicek, using Harris and Raymond's cyanogen bromide-para-aminoacetophenone method, found low nicotinic acid values for egg-white and milk and high values for all animal tissues, the highest of which were found in the liver and the adrenals. He also found high values for several cereals but suggested that this

was due to some interfering substance. (39)

Elvehjem and his co-workers used a biological assay of nicotinic acid content of foodstuffs by determining the amounts of the various materials needed to cure black tongue in dogs on a modified Goldberger diet. They found, in agreement with most of the more recent colorimetric methods of determination, that meats, liver extracts, and yeast were high in nicotinic acid and that wheat-germ, eggs, and milk were low in this substance. (40)

In January, 1941, Bacharach reviewed the results obtained with all the various types of determinations of the nicotinic acid content of foods used by various groups. He found a general consensus of high values in yeast, liver, lean meat, fish, unpolished rice, and peanuts; moderately high values in soy-bean, wheat, and barley; and low values in other cereals, dairy products, and eggs. Very complete and lengthy tables are published with this review. (41)

#### In Animal Material--Normal and Pathological

Investigation of the nicotinic acid content of animal tissues has been spurred on chiefly by the desire to find a method for the detection of beginning or subclinical nicotinic acid deficiency. Most work has been done, therefore, on the blood and urine.

The normal blood values vary a great deal with the procedure used. Swaminathan (29) reported .33 to .35 mgm. nicotinic acid per 100 cc. of human blood as normal; Kochar, using the same method, reported an average value of .367 mgm.% in normal human blood. (42)

Melnick, Robinson, and Field found an average blood level of .73 mgm.%. (43) Reports from various other workers range anywhere between these sets of figures. The average blood level for a group of normal persons as determined by Dr. Morgulis and me was .558 mgm.%, with values ranging from a low of .455 mgm.% to a high of .648 mgm.%, although most values were very close to the average (within about .05 mgm.%).

Melnick and his co-workers found that 90% of the total blood nicotinic acid is present in the erythrocytes. This group reported high values in polycythemia, presumably because of the high concentration of erythrocytes. This group also investigated the effect of various other factors on the blood nicotinic acid level. They found no appreciable effect on the blood level following recently-ingested meals, coffee-drinking, or smoking. They also found that a large oral dose of nicotinic acid caused a rise in the blood level, followed by a rapid drop to normal levels. Repeated doses of nicotinic acid caused an increase in the blood level which persisted for a long time after the dosage was stopped. (43)

No one has found any significant relationship between nicotinic acid blood levels and the state of nutrition. Kochar found pellagrins to have the same average as normal persons, and he found no rise in the blood levels on treating these patients with nicotinic acid, although they improved clinically. (42) Using the Euler-Myrback yeast fermentation method Axelrod, Madden, and Elvehjem could find no decrease in the coenzyme I content of blood, kidney cortex,

or brain in dogs and pigs in whom nicotinic acid deficiency had been produced by use of a modified Goldberger diet. They did, however, find that the coenzyme content of both liver and muscle was markedly decreased below the normal in these animals.(44) Axelrod, Elvehjem, and Spies found a similar situation in human pellagrins. The coenzyme I content of the erythrocytes was normal in these pellagrins but was found to be low in muscle tissue.(45)

Vilter, Vilter, and Spies reported in 1939 that the V factor (by the method of Kohn mentioned previously) was markedly below normal in the blood of pellagrins and severe diabetics (especially those with acidosis).(46) This is the only report in the literature of low blood values of either nicotinic acid or coenzyme in pellagrins. There is, furthermore, some overlap in the blood values of the pellagrous group and those of the normal group in this report.

Reports on normal urinary values for "nicotinic acid" are at great variance, chiefly because some methods determine only actual free nicotinic acid, some determine all types of pyridine derivatives except trigonelline, and others determine various numbers of compounds between these extremes. Swaminathan reports a daily excretion of 3 to 5 mgm. of nicotinic acid as determined by his method.(29) Harris and Raymond report the same values by their method.(32) Perlzweig, Levy, and Sarett found the normal daily excretion of all pyridine derivatives except trigonelline to be 1 to 3 mgm., and that of trigonelline to be about 20 to 28 mgm. These authors

found that coffee-drinking increased the trigonelline excretion to as high as 200 mgm. daily. (47)

There are several reports of finding low nicotinic acid levels in the urine of human pellagrins and of animals with experimental nicotinic acid deficiency. Vilter, Spies, and Mathews reported low urinary values in pellagrins by their method in 1938. (27) In 1939 Harris and Raymond found that dogs and guinea pigs deprived of nicotinic acid in their diet showed a gradual drop in the excretion of nicotinic acid which paralleled their clinical condition, until finally they excreted no nicotinic acid at all when in a state of severe deficiency. (32) Sarett has also very recently reported that the urinary excretion of acid-hydrolyzable nicotinic acid derivatives and of trigonelline fell to very low values in dogs in whom black tongue was produced experimentally. (48)

Field and his associates reviewed the literature on the chemical diagnosis of nicotinic acid deficiency in July, 1941, and found no definite relationship between the blood levels of nicotinic acid or of coenzyme in normal and in deficient individuals. They also had little faith in urinary determinations at present, for they stated that some fraction of nicotine is determined in nicotinic acid methods, and further, trigonelline, the chief excretory product of nicotinic acid, is also found as such in some foods, in coffee, and as a result of detoxification of nicotine in the body. They therefore feel that the chemical diagnosis of nicotinic acid deficiency is thus far wholly unsatisfactory. (49)

Sarett reported in January, 1942, that although dogs on high nicotinic acid diets excreted (as trigonelline and other derivatives) almost 100% of doses of nicotinic acid given orally, black tongue dogs did not excrete any part of these doses until several doses had been given, and even then only excreted part of the doses. (48) This discovery was applied to humans by Perlzweig, Sarett, and Margolis, who published a preliminary report in January, 1942, stating that they found hospital patients and undernourished youths excreted a smaller portion of a large dose of nicotinamide given orally than did normal persons. They suggest this as a possible method for detecting subclinical levels of nicotinic acid deficiency in man. Further investigation of the subject will be necessary before full evaluation of the method is possible. (50)

BIOCHEMICAL-PHYSIOLOGICAL SIGNIFICANCE,  
FATE IN THE BODY, AND REQUIREMENTS

To tell the story of the role nicotinic acid plays in the body economy we must go back to 1906, when Harden and Young reported their discovery that there was some substance in yeast which, after boiling and filtering or dialyzing, could enhance the fermenting action of yeast, although it could not initiate such fermentation by itself. The term cozymase was applied to this crystalloidal, thermostable substance which was so necessary for the process of yeast fermentation. (51)

Although much work was done on cozymase in succeeding years, it was not until this compound and a closely similar compound were isolated from animal tissues and found to contain nicotinamide in 1935 and 1936 that the role of nicotinic acid in the biochemical-physiological processes of the body began to be realized. The first of these substances to be isolated was not identical with Harden and Young's cozymase but was a closely similar compound which was found to be necessary for the oxidation of glucose-6-phosphate by an enzyme of mammalian erythrocytes. This substance was isolated in 1935 by Warburg and Christian, who called it coenzyme and characterized it as containing one molecule of adenine, one of nicotinamide, three of phosphoric acid, and two of ribose. (52,53)

About a year later Harden and Young's cozymase was isolated independently by Warburg and Christian (54) and by von Euler, Albers, and Schlenk. (55) This compound was also called a coenzyme,



and, since it was discovered first, was called coenzyme I, while the coenzyme isolated by Warburg and Christian in 1935 was called coenzyme II. Coenzyme I contains the same units as coenzyme II, except that it has one less molecule of phosphoric acid. Coenzyme I is also known as cozymase, diphosphopyridinenucleotide, and DPN. Coenzyme II is also called triphosphopyridinenucleotide and TPN.

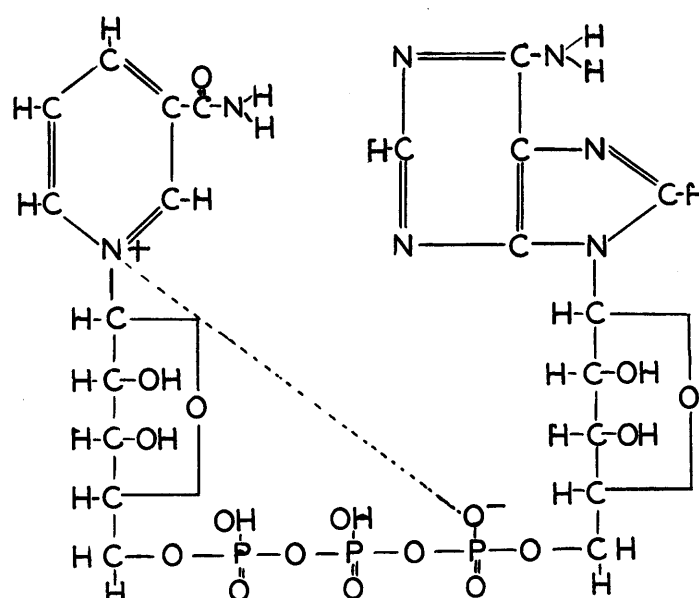


Fig. 3.--Structural Formula of Triphosphopyridinenucleotide

Although the structural formulas of these two important substances has not been proved definitely, they probably exist as shown in figure 3. The formula shown is that of triphosphopyridinenucleotide; diphosphopyridinenucleotide is exactly the same structurally, but it has one less esterified phosphoric acid group.

As intimated before, the importance of these coenzymes lies in the fact that they are necessary for the proper catalytic action

of certain enzymes. Just what part they play was first indicated by Warburg and Christian in 1935 when they proved that triphosphopyridinenucleotide constituted a reversible oxidation-reduction system and that nicotinic acid amide was the portion which was actually concerned in this process. They showed that in the oxidation of a substrate the nicotinamide group was reduced, the reduction taking place at the carbon-nitrogen linkage of the pyridine ring, the quaternary nitrogen then becoming a tertiary nitrogen. (53) The same type of reaction takes place in the case of diphosphopyridinenucleotide in its role as a coenzyme. This reaction is represented in figure 4, the formulas being simplified to show only the groups concerned in the oxidation-reduction reaction.

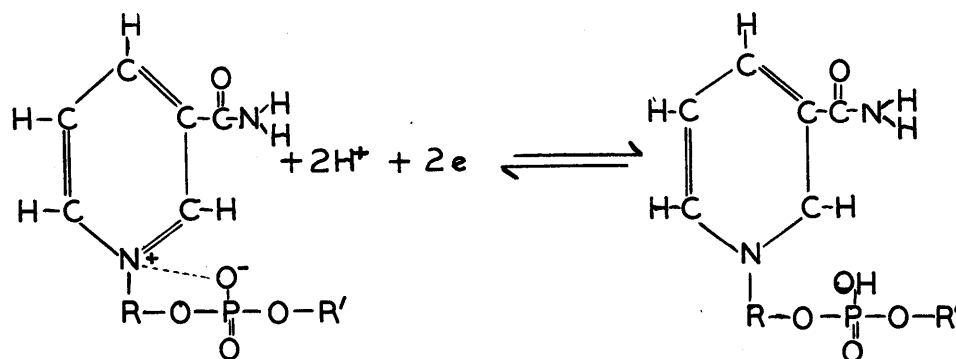


Fig. 4.--Mechanism of Oxidation-Reduction of Pyridinenucleotide

These two coenzymes play an essential role in some of the most important oxidation reactions in the body. Each reaction in which they take part depends on a protein enzyme which is specific for the substrate being acted upon. The protein combines with both the coenzyme and the substrate. The substrate is oxidized and the

coenzyme reduced; the protein remains unchanged.

A typical example of such a reaction is represented in the oxidation of triosephosphate by diphosphopyridinenucleotide. (Fig. 5) In this case the original substrate is hexose-diphosphate, which is divided into two triosephosphates by enzymatic fission. The aldose form reacts with diphosphopyridinenucleotide in the presence of a specific protein enzyme, resulting in reduced DPN and phosphoglyceric acid. This is one of the important steps in carbohydrate metabolism in the body. (Note: The symbol  $H_2$  when prefixing DPN or other enzymes in the following figures indicates the reduced form of the compound.)

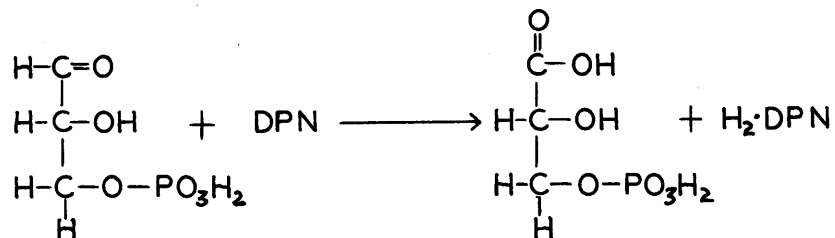


Fig. 5.--Role of Diphosphopyridinenucleotide in the Oxidation of Triosephosphate

An interesting connection with vitamin  $B_1$  (thiamine chloride) is now encountered. The phosphoglyceric acid produced in the above reaction is rearranged enzymatically to phosphopyruvic acid. This is then de-phosphorylated and the resulting pyruvic acid is further oxidized by means of cocarboxylase (diphosphothiamine, the essential group of which is thiamine or  $B_1$ ) to  $\text{CO}_2$  and  $\text{H}_2\text{O}$ .

The coenzyme oxidation-reduction is a reversible reaction, but reduced coenzyme cannot be oxidized by air. The reduced coenzyme

is oxidized by flavoprotein (the essential part of which is riboflavin) forming reduced flavoprotein, which in turn is oxidized by the oxygen of air through the medium of cytochrome. (Fig. 6) The same reaction is necessary to oxidize reduced cocarboxylase to its active form. Thus we see that three of the main components of the B-complex of vitamins, namely, nicotinic acid, thiamine, and riboflavin, are intimately concerned with each other in biological oxidations.

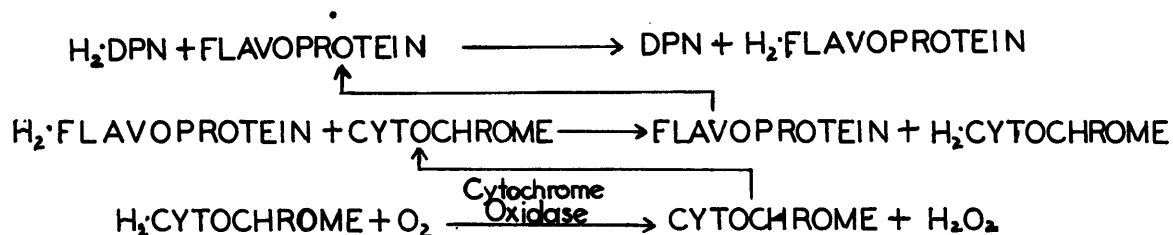


Fig. 6.--Mechanism of Hydrogen Transfer and the Re-oxidation of Carriers

The coenzymes play an important part not only in carbohydrate metabolism, as indicated above, but also in protein metabolism. The reaction is essentially the same in this case as that for the oxidation of carbohydrate. The amino group of the amino-acid is oxidized to an imino group, while the coenzyme is reduced. Reoxidation of the coenzyme again occurs as described before, so that it may enter the reaction again. Glutamic acid is the only amino-acid thus far proved to be oxidized by coenzyme. (56,57)

Although the above statements suggest that the coenzymes do not need to be replaced since they may be used over and over again,

this is not the case; for some reason they are gradually depleted in reacting, and new coenzyme must be synthesized from the various components, especially the nicotinamide. That such synthesis actually takes place was first proved by Kohn and Klein in 1939. They showed that the content of cozymase and of factor V in human erythrocytes was increased by the oral administration of nicotinic acid in normal humans and by the incubation of defibrinated blood with nicotinic acid in vitro. (58) Vilter, Vilter, and Spies confirmed this in their work on pellagrins. They also found that injection of riboflavin into these patients caused a further marked rise in the V factor of the erythrocytes, above and beyond that produced by nicotinic acid alone. They considered that riboflavin probably assists in the synthesis of the coenzymes from nicotinic acid. (46) Axelrod, Spies, and Elvehjem also found that administration of nicotinic acid to pellagrins caused a marked rise in the coenzyme content of erythrocytes and muscle. (45)

Although the two coenzymes are so closely related chemically and so identical in their mode of action, they react only in the oxidation of certain specific substrates. Triphosphopyridinenucleotide alone can act as the coenzyme in the oxidation of hexosemonophosphate, citrate, and phosphohexonate. Only diphosphopyridinenucleotide, on the other hand, can act as the coenzyme in the oxidation of lactate, malate, beta-hydroxybutyrate, aldehyde (mitase), triosephosphate, glycerophosphate, and glyceraldehyde. Either coenzyme is apparently effective in the oxidation of glucose

or of glutamic acid in the liver. (56,57)

From the above discussion it is seen that nicotinic acid plays a definitely-known role in body metabolism. However, the manner in which nicotinic acid protects the skin, gastro-intestinal tract, mucous membranes, and brain from developing the lesions seen in pellagra is as yet unknown. The final story of nicotinic acid will not be told until this problem is solved.

The fate of excess nicotinic acid in the body was first investigated by Ackermann in 1912. (59) He found that the chief excretory products of nicotinic acid were trigonelline, the methyl betaine of nicotinic acid, and nicotinuric acid, the dipeptide of glycine and nicotinic acid. (Fig. 7) Melnick, Robinson, and Field investigated

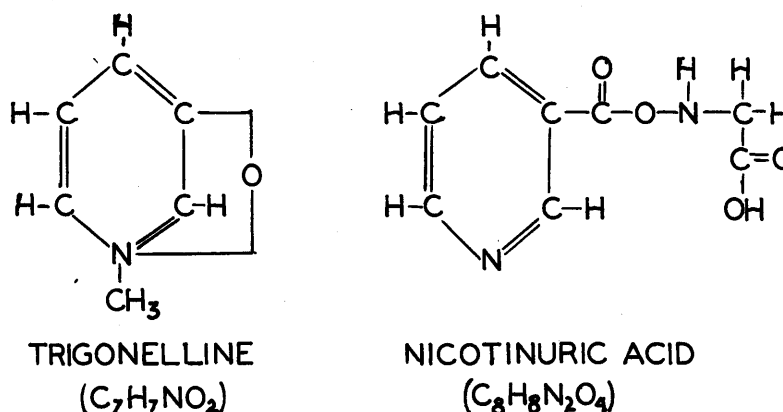


Fig. 7.--Excretory Products of Nicotinic Acid

the excretory products of nicotinic acid by feeding normal persons large doses of nicotinic acid. They found a great increase in the "pyridine bodies" of the urine, mostly in the first hour after ingestion of the nicotinic acid, gradually decreasing in the next

three hours. Of this increased excretion of pyridine compounds, 51% was trigonelline, 30% nicotimuric acid, and 13% free nicotinic acid. (60)

Although nicotinic acid has been definitely proved to be a necessary factor for proper nutrition in the human, the dog(17), the pig(61), and the monkey(62), no one has yet worked out definitely the actual quantitative requirements for any of these species. It has been proved that sheep(63) and rats(64) do not need nicotinic acid in their diet, for they are able to synthesize this factor by some as yet unknown means.

It is known, however, that the requirement for nicotinic acid is increased by anything increasing carbohydrate metabolism, probably through accelerated depletion of coenzymes. Factors causing such an increased need would be pregnancy, acute hyperthyroidism, fever, excess physical exertion, maintenance on intravenous glucose for many days, or sudden adding of large increments of carbohydrates and insulin to a diabetic diet. The requirement would also be increased by any condition causing faulty absorption of nicotinic acid, as vomiting, diarrhea, achlorhydria, or edema of the gastrointestinal mucosa due to cardiac failure, renal damage, or portal obstruction. Faulty utilization of nicotinic acid, as in portal cirrhosis, fatty degeneration of the liver, or passive congestion of the liver, would likewise increase the requirement of the individual for nicotinic acid. (74)

## PHARMACOLOGY AND TOXICITY

Since nicotinic acid is being used very extensively in therapy, it is desirable to know something of the actions of the drug on the body functions and also its toxic effects, if any. Chen, Rose, and Robbins investigated the toxicity of nicotinic acid soon after it was first used in the treatment of human pellagra. They found the MLD for rats and guinea pigs to be about 3.5 to 4.5 grams per kilogram of body weight. They also found that oral administration of 2 grams of nicotinic acid daily to dogs caused death or severe illness. Administration of less than 1 gram was apparently not harmful in any way. (65)

Some time later Unna reported the results of his studies on the effects of nicotinic acid on rats, chickens, and dogs. He found no evidence of toxic symptoms during life or of pathological changes post-mortem in any of the organs, although these animals were fed as much as 2 grams of nicotinamide per kilogram body weight daily for periods of up to two months. There was no effect noted on the metabolism, the circulatory system, or the respiratory system of normal animals given nicotinic acid or its amide. A slight transient rise in the blood pressure was thought to be due to the acid pH of the solution used. (66)

In treating pellagrins with nicotinic acid Fouts et al (18) and Spies and his associates (20) found that their patients experienced sensations of heat, tingling, itching, and flushing of the



skin, especially of the face and extremities. The effect was transitory, however. Spies and his co-workers investigated these effects more thoroughly and found that the administration of 200 mgm. of nicotinic acid orally or of 10 mgm. intravenously was followed in one minute by dilatation of the small vessels of the skin of the face and upper trunk. The subjects complained of heat, flushing, burning, and itching sensations. There was some increased activity of the sebaceous glands and increased motility of the gastro-intestinal tract. There was no effect on the blood pressure, respiratory depth or rate, pulse rate or rhythm, or the electrocardiographic record. These workers felt that nicotinic acid has a weak histamine-like action. (67)

Scaffidi (68) and Cardin (69) have found that nicotinic acid causes increased tone and increased contractions of isolated portions of stomach and intestine of guinea pigs and rabbits. This effect is enhanced by atropine, (68) but it is not produced by the use of nicotinamide or sodium nicotinate. (69)

Sydenstriker et al found in treating pellagrins with nicotinic acid that there was an increase in the secretion of gastric hydrochloric acid, even in some cases of achlorhydria. They confirmed this effect by giving nicotinic acid to normal persons with a resultant increase in the gastric acidity. (70) Malaguzzi and his co-workers stated that injection of nicotinic acid into normal persons produced as great a rise in gastric hydrochloric acid as did the injection of histamine but failed to give rise to any secretion

of acid in cases with complete achylia. (71)

Moore proved by means of exposing the blood vessels of the spinal canal and of the brain in laboratory animals and by measuring the intraspinal fluid pressure on humans that nicotinic acid caused vasodilatation of the brain and spinal cord, as well as of the skin. (72)

Bean and Spies have reported that additions to the pyridine ring of nicotinic acid or removal or aminization of the carboxyl group made the substance inert as a vasodilator. Food taken just before the nicotinic acid also prevented its unfavorable vasodilating effect. These workers suggest that repeated small doses of nicotinic acid or use of some derivative of nicotinic acid will eliminate any unpleasant side effects. (73)

## CLINICAL USES

In Pellagra

"Pellagra is a disease entity characterized pathologically by an erythematous, pigmented, and exfoliative dermatitis; stomatitis; glossitis; gastro-enteritis; hepatitis; proctitis; urethritis and vaginitis; and clinically by the subjective symptom syndrome consisting of burning ("scalded") sensation in the mouth and tongue, anorexia, diarrhea, burning in anal region and in the urethra, or vagina; varied nervous manifestations; emaciation, cachexia, and varying degrees of anemia from the mild secondary type to the blood picture of severe primary anemia."(4)

The classical triad of symptoms in pellagra is dermatitis, diarrhea, and dementia. However, all of these need not be present in every case of pellagra. Although the dermatitis is considered especially characteristic, many cases have been reported with no skin lesions demonstrable, the so-called pellagra sine pellagra.

Sydenstricker describes two main types of pellagra, one a result of long-continued mild deficiency, the other the result of a severe, rapid depletion of nicotinic acid (as in severe alcoholism or in maintenance for long periods on intravenous glucose). In the chronic partial deficiency the first symptoms are usually mild psychic disturbances. Next vague gastro-intestinal disturbances with anorexia and mild glossitis make their appearance. As this continues we get achlorhydria, diarrhea, and more severe psychic

disturbances. Dermatitis may appear at any time. The patient may get infections in the mouth, vagina, rectum, or esophagus as a result of the lowering of the cellular defense mechanisms. Finally we get delirium, stupor, dementia, or an encephalopathic syndrome. In severe cases there may be irreversible changes in the gastrointestinal mucosa and in the brain (with permanent dementia). In the severe rapid deficiency we find marked psychic disturbances, severe glossitis (often accompanied by infection with Vincent's organisms or fungi), and occasionally bullous or necrotizing dermatitis. (74)

This disease was once very difficult to cure. Although dietary treatment had been used almost since the time of Casal's first description of pellagra, the prognosis in most cases was poor, and mortality rates, especially in institutions, were fearfully high. However, since Elvehjem's discovery that nicotinic acid was the pellagra-preventive factor in 1937, reports of almost miraculous cures of all types of pellagra by nicotinic acid have been published, and the mortality rate from this disease has dropped steadily to very low figures. Fouts et al (18), Smith, Ruffin, and Smith (19), Spies and his associates (20,67), and Sydenstriker and his co-workers (70) reported cures of large numbers of cases of human pellagra within a few months after Elvehjem's original report was published. Since that time innumerable reports of cures of pellagra by using nicotinic acid or some of its derivatives have been published.

The amount of nicotinic acid necessary for the cure of pellagra

has not been established. Fouts and co-workers used 500 mgm. daily with good results. (18) Sydenstricker recommends from 300 to 1800 mgm. of nicotinic acid daily depending on the severity of the condition, with decrease to 100 to 150 mgm. daily after improvement is seen. (74) From all reports it appears that at present the best guide in therapy is the clinical condition of the patient. Therapy should not be stopped because of the transitory unpleasant effects of flushing, tingling, or burning of the skin.

The fact that all symptoms may not be relieved by nicotinic acid alone brings out the connection of pellagra with deficiency of other vitamins, as stressed by almost all authors. It has been suggested, because of this fact, that all patients receive large doses of all vitamins, especially the B-vitamins, as well as a high-caloric, well-rounded diet. Liver or liver extracts have been especially recommended for such a purpose. (4)

Elvehjem has stated that nicotinic acid should be used in the treatment of pellagra solely as an emergency measure. Since the basic cause of the condition is dietary, he suggests, for permanent cure, correction of the diet by use of proper foods, high in vitamins, proteins, and other essential elements. Special care must be used in areas where pellagra is endemic to provide diets high in nicotinic acid. (75)

#### In Gastro-intestinal Disorders

Although it has long been known that gastro-intestinal pathology

exists in pellagra, it has been only within the past twenty years or so that a concept has arisen suggesting that pellagra may actually be a result rather than a cause of gastro-intestinal pathology in many cases. The first report of pellagra following established preceding pathology of the gastro-intestinal tract was that of Rolph in 1916, who reported classical pellagra in a woman with carcinoma of the stomach.(76) In 1925 Joyce and Seabrook reported a case of pellagra following the development of stricture of the rectum.(77).

In 1926 O'Leary first suggested the term "secondary pellagra" to denote cases of pellagra following organic pathology of the gastro-intestinal tract. He reported several cases of pellagra in patients with obstructing lesions of the stomach or intestine.(78). Cases of pellagra developing in patients with ulcerative colitis were reported by Barnes in 1926 (79) and by Jankelson and McClure in 1940.(80) In Barnes' case the patient was actually under hospital care with a high-caloric, low-residue diet at the time pellagra developed.

Turner reported 25 cases of secondary pellagra in 1929. He found that these comprised about 20% of all pellagra cases seen. By far the greatest number of cases were secondary to rectal stricture. Others had gastric carcinoma, ulcerative colitis, and several other miscellaneous organic disorders. Several of these patients had been on what was considered adequate diets before they developed pellagra.(81) Thaysen reviewed the literature on secondary pellagra in 1932 and found that most cases were caused by stenosis

of the digestive tract, with the next most frequent cause being alternating diarrhea and constipation, and the next most common cause diarrhea alone. (82)

There has been a good deal of work done on the occurrence of achlorhydria in pellagra. Thaysen finds in his review that 87% of the cases had low or absent gastric acid. (82) Sydenstriker reports complete achlorhydria in about 80% of his cases, with low gastric acidity in almost all of the remaining 20%. (83) Tommaseo reports the gastric acidity as being below normal in all cases of pellagrous children studied. (84) Diaz-Rubio reports in studying 342 cases of pellagra that 95% showed no free acid and low total acid, 70% had blood, 75% had lactic acid, and 40% had large amounts of mucus in the gastric contents. (85)

After nicotinic acid was shown to be the pellagra-preventive factor, the response of cases such as those described above to this compound was investigated. In 1938 Hanssen reported that a case of pellagra occurring six months after sub-total gastric resection was treated successfully with campolon (a liver extract). (86) In 1939 Massen reported a case of pellagra secondary to gastro-enterostomy which was not affected by liver extract and B<sub>1</sub>, diet, or other treatment, but responded promptly to the use of nicotinic acid. (87) Sydenstriker (70), Tommaseo (84), and Diaz-Rubio (85), showed that nicotinic acid increased the gastric secretion in pellagrins, even in some cases of complete achlorhydria. Malaguzzi, however, reported that although the atrophic glossitis was improved

in complete achylia cases, there was no rise in the gastric acid after the administration of nicotinic acid. However, these were not pellagrous achlorhydria cases. (71)

There has not been much investigation on the effect of nicotinic acid on the gastro-intestinal functions aside from that on the effect on the secretion of gastric acid. Crandall, Chesley, Hansen, and Dunbar found definite changes in the gastro-intestinal tract of dogs on a black tongue-producing diet by the use of X-ray studies. They found first hypermotility (appearing in about three to five weeks); then areas of atonic bowel, with "puddling" of the barium in these areas, and gas in the small intestine were next observed. The large bowel was least susceptible to these changes, but eventually the entire gastro-intestinal tract was affected. These changes all appeared before any signs of black tongue could be discerned in all but one dog. (88) In 1940 Bean and Spies found, by means of fluoroscopic studies on humans, that nicotinic acid caused increased peristalsis of the intestinal tract, with greater tonus and greater contraction waves than before the injection of the drug. (89)

Some very interesting experiments have been carried out by Petri, Nørgaard, and Bandier on pigs. They have performed total gastrectomies on several groups of pigs. After recovery these groups were placed on different diets. Some were given nicotinic acid; some were deprived of this substance in their diet; others were given various vitamins, alone or in combination, with their diet.



All groups developed pig pellagra in about the same time--6½ months after gastrectomy. Furthermore, the various vitamins, including nicotinic acid, had no effect even if given parenterally. The obvious conclusion, therefore, is that some gastric function is necessary for the prevention of pellagra in pigs. Just what this factor or function is has not been ascertained. Whether some similar mechanism is necessary in other animals and humans is also unknown, but investigation of the subject would certainly be worth-while.

(90-91)

There have been reports of the use of nicotinic acid in gastrointestinal pathology exclusive of that associated with definite pellagra. Bing and Broeger (92) and Fuchs and Wisselinck (93) report improvement of several cases of sprue on treatment with nicotinic acid alone. Siedek and Reuss report a case of severe diarrhea resembling sprue in which the diarrhea was controlled and the stools became fat-free on treatment with nicotinamide.(94) Teasley reports the dramatic cure of several cases of summer diarrhea of the non-infectious type in infants with no other medication than vitamins, especially nicotinic acid.(95) Spies et al report several cases of constipation without pellagra in which normal function returned after treatment with nicotinic acid. Some other cases of the same group, however, failed to respond to this treatment.(67)

Borsook and his associates reported a very large series of patients with gastro-intestinal complaints without demonstrable organic lesions who were relieved of their symptoms by the use of

B-complex vitamins in the form of rice-polishing concentrate or special wheat cereal, where merely adequate dietary regime had failed. There were no signs of beri-beri or pellagra in any of these cases. (96) Chesley, Dunbar, and Crandall also report a large series of cases with gastro-intestinal complaints (chiefly flatulence, alternate constipation and diarrhea, and abdominal distress) in whom no organic lesion could be found. Whole vitamin B complex or nicotinic acid alone relieved the symptoms, whereas thiamine and riboflavin alone failed to do so. These workers suggest better response to whole B-complex than to nicotinic acid alone, however. (97)

From the above discussion it can be readily seen that the role of nicotinic acid in treatment of gastro-intestinal disease is far from being established. For the most part the literature contains unrelated reports, most of which are unconfirmed, so that no clear picture of the whole subject can yet be drawn. In this field of nicotinic acid therapy thorough investigation is indeed sorely needed.

In Neurological Diseases

Mental symptoms have been known to be a part of the pellagra syndrome since the earliest recognition of the disease. Some interesting names which have been given to pellagra by the Spanish and the Italian peasants in the past bring out this fact. The disease has been called mal del higado (disease of madness), mal della spienza (disease of melancholia), and calore del fegato (the heat

or fever of madness). More recently Italian physicians have called it psychoneurosis maidica (the psychoneurosis of corn or maize). (98)

In 1913 Singer and Pollock investigated the central nervous system pathology in pellagra very thoroughly. They found in the acute attack of pellagra a picture of "central neuritis", as seen in general intoxications. There was also chromatolysis of neurons, satellitosis, astrocytosis, presence of amoeboid glial cells, and moderate perivascular infiltration. In chronic cases they found in the central nervous system fatty and fibrinoid degeneration, chronic Nissl body changes in the cells, destruction of nerve fibers, increase of glial fibers, but no vascular changes. (99)

Spies et al reported in 1938 that the mental symptoms of pellagra were usually of the so-called neurotic type, especially what is classed as neurasthenia, characterized by lassitude, palpitation, numbness, fatigue, and other vague symptoms. Some patients showed delusions, disorientation, depression, or memory loss. All mental symptoms were cured promptly by the use of nicotinic acid. (100)

Matthews reported similar cases with the same results at about the same time. He mentions delusions, hallucinations, with sometimes depression and even stupor as the most common symptoms in his patients. (101)

Frostig and Spies re-investigated the mental symptoms associated with pellagra in 1940 and suggested that the initial nervous symptoms were general hyperesthesia, increased psycho-motor drive, fatiguability and weariness, headaches, and sleeplessness. In

general they report that the picture is one of an anxiety state with depression. Although symptoms are cured promptly with nicotinic acid alone, these authors suggest a well-balanced diet be given as well. (102)

Sydenstriker and his associates were the first to attribute certain mental symptoms to nicotinic acid deficiency when none of the usual signs of pellagra were present. The cases reported usually showed stupor or clouding of consciousness. Some cases showed irrational behavior, delusions, disorientation, and other signs of what is usually called toxic psychosis or exhaustion delirium. All cases improved on treatment with nicotinic acid although some few required doses of over 1000 mgm. daily. All but a few cases were completely cured. (103,104)

More recently Jolliffe and his co-workers have reported 150 cases of an encephalopathic syndrome characterized by clouding of consciousness, cog-wheel rigidities, and uncontrollable grasping and sucking reflexes. On a treatment of hydration alone or hydration plus thiamine chloride, 95 to 100% died; on a treatment of hydration plus whole vitamin B complex there was a drop in mortality to 62%; but on hydration plus nicotinic acid alone the mortality dropped to 32%, and since most of these cases died of some intercurrent infection, the "corrected mortality" on nicotinic acid therapy was about 14%. These workers call the syndrome "nicotinic acid deficiency encephalopathy" on the basis of these findings. No signs of pellagra were present in the group of patients. (105,106)

Other uses of nicotinic acid in neurological disorders include its use in acute alcoholic syndromes. Mainzer and Krause reported cure of a case of delirium tremens with nicotinic acid where thiamine treatment had failed. This patient also had some stomatitis, vomiting, and alternate diarrhea and constipation. (107) May reported the cure of four cases of alcoholic psychosis by giving 600 mgm. of nicotinic acid daily. (108) Most neurologists feel that nicotinic acid should be given along with thiamine chloride in the various alcoholic psychoses.

Reports of the use of nicotinic acid in motor tract involvements are not very numerous. Blankenhorn reported cure of two cases of spastic paralysis with pellagrous signs following alcohol addiction by the use of brewer's yeast and a diet high in calories, vitamins, and proteins. (The treatment was carried out in 1937, before nicotinic acid had been identified as the "P-P factor"). (109) Moore reported marked improvement in five cases of multiple sclerosis on treatment with nicotinic acid and thiamine. He attributed the effect to the vasodilating action of nicotinic acid on the vessels of the central nervous system, such as occurs in fever therapy, which is often used successfully in multiple sclerosis. (72)

#### Miscellaneous Uses

There have been so many different miscellaneous uses suggested for nicotinic acid in the literature that it is difficult to see how all of them can be valid. Only those which have been fairly

well accepted will be mentioned.

The use of nicotinic acid in Vincent's angina was first suggested by the constant presence of stomatitis in pellagra. In 1939 Morris and Franklin reported improvement in dogs suffering from fuso-spirochetal stomatitis after the administration of nicotinic acid. (110) King reported cure of four human cases of severe ulceration of the mouth associated with the proved presence of Vincent's symbiotic organisms. This cure was accomplished solely by the administration of nicotinic acid with no local treatment. (111)

Harris and Moore (112) and Atkinson (113) have reported definite improvement of symptoms in patients with Meniere's syndrome on treatment with nicotinic acid, either alone or combined with thiamine. These cases had no closure of the Eustachian tube and no intralabyrinthine hemorrhage as the basis for their syndrome. Cases with such etiologic factors could not be expected to respond to nicotinic acid. (112) The results were attributed to the vasodilating action of the nicotinic acid. Young patients showed the most improvement. (113)

Numerous other uses of nicotinic acid have been reported. Saphir reported prompt cure of xerostomia with nicotinic acid plus yeast and thiamine, whereas the treatment was not successful with only the last two. (114) Graham reported marked alleviation of symptoms of radiation sickness by the use of nicotinic acid. (115) Selfridge has reported success in the treatment of high-tone deafness with nicotinic acid. (116) Most of these reported uses of nicotinic acid require further investigation before definite conclusions may be drawn.

## SUMMARY AND CONCLUSIONS

There seems to be no division of opinion about the efficacy of nicotinic acid in the treatment of pellagra. Suggestions have been made by most authors, however, that other vitamins, especially of the B complex, must also be supplied for maximum curative effect. Furthermore, correction of dietary factors to prevent the disease or relapses is considered essential.

As to the other uses of nicotinic acid there is no real consensus of opinion. However, most psychiatrists recommend its use with thiamine chloride in the various toxic psychoses, especially those of alcoholic origin. Use of the drug in vague gastro-intestinal complaint, in peculiar neuro-psychiatric syndromes, and in various miscellaneous conditions is still questionable and must remain so until a greater mass of reports is available.

Chemical diagnosis of nicotinic acid deficiency is at present unsatisfactory, although there are several good methods which could be used clinically if suitable criteria can be worked out.

As has been stated in the body of this paper, although the role of nicotinic acid in biological oxidations is known, its manner of protecting the skin, mucous membranes, gastro-intestinal tract, and central nervous system is still unknown, and until we do know something of this activity, we can not use nicotinic acid to its greatest advantage in the practice of medicine.

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