THE BIOLOGICAL SIGNIFICANCE OF NICOTINIC ACID

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IF we review briefly the developments in the field of biochemistry, we find that chemical elements and chemical compounds have become biologically significant by diverse pathways. The various methods by which the five recognized members of the B complex have come to be known as specific chemical compounds makes an interesting story in itself. In the case of riboflavin the new compound was isolated and later associated with fundamental processes in the living cell. In the other cases the necessity of compounds of specific character was recognized first and finally the actual compounds were isolated and identified. Thiamin, vitamin B_6, and pantothenic acid turned out to be compounds of rather unique structure, quite different from those generally found in living matter. On the other hand, the antipellagra factor was found to be a compound which had been known for many years.

It is quite obvious that in all cases our knowledge of the biological significance of these factors has increased more rapidly after the compounds became available in pure form. Thus we are not so interested in the manner in which the significance was recognized as we are in the entire and complete picture. It is my purpose this evening to give you as complete a picture as I can of the facts concerning the third member of the vitamin B complex—nicotinic acid.

Huber[1] first prepared nicotinic acid in 1867 by the oxidation of nicotine. Its isolation from biological material was not achieved until 45 years later when Funk[2] isolated nicotinic acid in crystalline form from yeast concentrates which possessed antineuritic activity. The acid itself, however, displayed no activity in curing pigeon beriberi. At about the same time Suzuki, Shimamura, and Odake[3] isolated nicotinic acid from rice polishings. In 1916 Williams,[4] impressed by the common occurrence

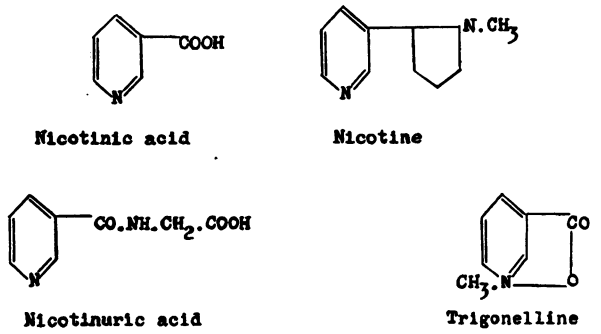

Figure 1

of nicotinic acid with the antineuritic vitamin in several natural substances, again tried nicotinic acid, trigonelline, as well as other pyridine derivatives for antineuritic potency, but none of them caused any permanent improvement in polyneuritic fowls. Trigonelline, the methyl betaine of nicotinic acid, was isolated from plant material by Jahns[5] in 1885. In 1912 Ackermann[6] found that dogs given fairly large amounts of nicotinic acid excreted in the urine about equal amounts of trigonelline and nicotinuric acid (the dipeptide of nicotinic acid and glycine). The formulæ for these compounds are given in Fig. 1.

Except for the work of Szymanska and Funk[7] who attributed an appetite-stimulating and weight-preserving action to nicotinic acid and the amide, very little interest was shown in the possible role of pyridine derivatives in living systems until the work of Warburg and Euler in 1935. Warburg and Christian[8] characterized nicotinic acid amide as one of the hydrolysis products from the coenzyme which they had isolated from blood and which is now known as coenzyme II. Kuhn and Vetter[9] isolated nicotinic acid amide from heart muscle and Euler, Albers, and Schlenk[10] from cozymase.

This work gave new impetus to the application of these compounds in the field of nutrition. Euler and Malmberg,[11] using a diet similar to the Sherman-Bourquin diet supplemented with vitamin B_1 and riboflavin, found no growth response with the acid or amide although the rats receiving the acid lived longer. Funk and Funk[12] found larger food intake and better growth in rats and pigeons on certain rations when given the acid and especially the amide. In our own laboratory[13] we observed a growth stimulus from nicotinic acid when fed with adenylic

acid to rats on a factor W deficient diet. However, none of these responses was of sufficient magnitude to attribute to nicotinic acid real vitamin-like properties.

Let us now turn our attention to the human disease, pellagra. Almost twenty years ago Dr. Voegtlin presented before this Society the studies on pellagra available at that time and concluded that the disease could be prevented by appropriate diet, but the nature of the dietary factor had not been discovered. By 1930, due to the excellent work of Goldberger and co-workers, pellagra had been definitely established as a deficiency disease and the protective factor was associated with the more heat stable fraction of the B complex which had been distinguished from vitamin B_1 by animal experimentation. A pellagra-like condition had been produced in rats on diets low in the B complex supplemented with an alcoholic extract of corn. I want to emphasize at this time that Goldberger never referred to this syndrome as anything except a pellagra-like condition. The symptoms observed in rats by Goldberger were undoubtedly those which we associate today with vitamin B_6 and riboflavin deficiency. Considerable difficulty was encountered in producing this condition consistently but the growth-stimulating effect of various foods when added to this diet continued to be used as a measure of antipellagra activity. All the values in the literature were based on these assays except the limited figures reported by Sebrell[14] from studies with dogs.

In 1930 the value of liver extract in the treatment of pellagra in humans as well as its activity in the prevention of vitamin B_2 deficiency in rats was recognized. Goldberger and Sebrell[15] found liver extract 343 to be a good source of the factor necessary for the prevention of black tongue in dogs. Spies[16] and Smith and Ruffin[17] found that fairly large amounts of liver extract by mouth were efficacious in treating pellagra. At about the same time I was working in the Biochemical Laboratory, Cambridge, England, and Dr. Guha, who was also working there, found the liver extract, which I had brought along for another purpose, produced excellent growth in rats on a vitamin B_2 deficient diet. The following year Salmon and Guerrant[18] found liver extract to be four times as rich in vitamin B (B_2) as a sample of brewers' yeast. We now know that the growth obtained in both cases must have been due mainly to the riboflavin present in the liver extract. However, the above results were sufficient to convince me that liver extract might be an excellent material for the isolation of the antipellagra factor.

Attempts in our laboratory to produce pellagra-like lesions in rats failed completely and we turned our attention to chicks. A pellagra-like condition was produced in the chick by feeding a heated natural grain ration, and the assays of our fractions from liver extract depended upon the prevention of these lesions. We[19] soon had the first indication that the factor active in this syndrome was separate from riboflavin which Kuhn, György, and Wagner-Jauregg[20] had just isolated and shown to have growth-promoting properties in rats. Concentrates of riboflavin were completely inactive for the chick while purified fractions from liver retained their potency after removal of riboflavin. Lepkovsky and Jukes[21] soon confirmed our experimental results and introduced the name "filtrate factor" for the active substance in the filtrate after removal of the riboflavin with fuller's earth. This designation did not exactly please us since naturally we felt that we were dealing with the true antipellagra factor, but both Dr. Lepkovsky and I recognized that there was no evidence available to show that the pellagra-like condition in chicks was identical with human pellagra. It was therefore necessary for us to repeat our work using dogs, since most authorities agreed that black tongue in dogs was identical with human pellagra. Again[22] riboflavin had no effect on black tongue while the purified concentrates free of riboflavin were highly active in curing this syndrome. Birch, György and Harris[23] confirmed the fact that riboflavin had no curative action in dogs and Dann,[24] Spies,[25] and Fouts, Lepkovsky, Helmer, and Jukes[26] reported complete inactivity of riboflavin in the treatment of human pellagra.

Further purification of the liver fractions gave concentrates which contained very small amounts of solid material and which were highly active in both chicks and dogs. It was not surprising that we believed that the chick and dog required the same factor. Within a very short period we[27] demonstrated the activity of nicotinic acid in the cure of black tongue, and isolated nicotinic acid amide from liver extract concentrates. The activity of nicotinic acid in the treatment of black tongue was soon verified by Street and Cowgill,[28] Dann,[29] and Sebrell and coworkers.[30] However, when we[31] tried nicotinic acid or the amide on chicks we found these compounds completely inactive in the prevention of the pellagra-like lesions in this species. The chicks had provided a means of assay not because we were actually testing for nicotinic acid but because we were testing for the chick antidermatitis factor which followed the nicotinic acid amide very closely. Recent work in our

laboratory conducted by Dr. Woolley has identified the chick antidermatitis factor as pantothenic acid.

The activity of nicotinic acid in the treatment of black tongue suggested its therapeutic use in human pellagra and its successful use has been reported by a number of workers in the field. The value of nicotinic acid has recently been reviewed by Spies, Bean, and Ashe[32]. They make the following summary concerning its use. "In cases of acute or chronic pellagra in relapse it will: (a) cause fading of the fiery red lesions of the mucous membranes and diminish the Vincent's infection associated with it, (b) in most cases, restore to normal disturbed gastrointestinal function, (c) restore to normal the mental function deranged moderately or severely in acute pellagra, (d) cause fading of the dermal erythema but not cure chronic changes of the skin. In cases of subclinical pellagra, the vague ill-defined symptoms disappear and in persons subject to recurrences of the disease the development of clinical pellagra is prevented. In both clinical and subclinical pellagra, the sense of well-being, one of the attributes of health, is restored."

Very recently Cleckley, Sydenstricker, and Geeslin[33] have reported the beneficial effect of nicotinic acid in the treatment of atypical psychotic states.

Katzenellenbogen[34] working in Palestine brought about considerable improvement in twenty-one out of twenty-four cases of stomatoglossitis, characterized by soreness of the tongue and angles of the mouth and sore throat. Landor,[35] however, could not cure stomatitis with nicotinic acid but did find yeast to be active. It is quite possible that these conditions may be related to the cheilosis which Sebrell[36] has shown to be due to a riboflavin deficiency.

Nicotinic acid has also been used in the treatment of disorders related to black tongue in dogs and certain intestinal disturbances in pigs. It is safe therefore to conclude that diets low in nicotinic acid or compounds which yield nicotinic acid on ingestion allow the development of pellagra and related disturbances in humans and similar conditions in dogs, pigs and monkeys.

The above results raise certain pertinent questions: (a) What is the distribution of nicotinic acid or nicotinic acid precursors in natural foods? (b) Are other compounds equally as effective as nicotinic acid, and (c) How does nicotinic acid function in the animal body?

The successful use of natural foods as a source of this vitamin de-

pends upon the availability of complete figures for its distribution in a large variety of foods. Possible methods for determining the distribution of nicotinic acid include chemical procedures, bacterial growth methods, and animal assays. The method of Karrer and Keller[37] based on the color produced with 2,4 dinitrochlorbenzene is definitely not reliable since the value given by Karrer for liver is one-tenth of the amount of nicotinic acid amide which we actually isolated from liver and we calculated that we recovered only one-twentieth of that present. The method which has been most satisfactory depends upon the breakdown of the pyridine nucleus by cyanogen bromide and aniline to give a yellow colored compound which can be measured colorimetrically. Swaminathan[38] has used this method on foods and Shaw and MacDonald[39] on commercial liver extracts. Bandier and Hald[40] have used p-methylaminophenol in place of aniline, and Bandier[41] has recently applied this method to biological material with satisfactory results.

Nicotinic acid was shown to be an important growth factor for Staphylococcus aureus by Knight,[42] for diphtheria bacillus by Mueller,[43] and for dysentery bacillus by Koser, Dorfman, and Saunders.[44] Although some of these methods have been used for the quantitative estimation of nicotinic acid in body fluids, no extensive studies have been made on foods. Similarly cozymase has been determined through the use of the bacilli of the influenzæ group as originally demonstrated by Lwoff and Lwoff but as yet this method has been applied mainly to blood by Vilter, Vilter, and Spies[45] and by Kohn[46].

As far as animal assays are concerned, both the rat and the chick are eliminated at least for the time being. We have, therefore, continued to rely upon the dog for our assays. The curative method has been used almost exclusively. Black tongue was produced on our regular basal ration containing:

Yellow corn	72
Purified casein	18
Cottonseed oil	5
Cod liver oil	2
$Ca_2(PO_4)_2$	1
$CaCO_3$	1
NaCl	1
Thiamin	50 gamma per K per day
Riboflavin	" " " " " "

TABLE I

NICOTINIC ACID POTENCY OF FOODS BASED ON BIOASSAYS WITH DOGS

Material	*Mg. per gm. dry weight*	*Material*	*Mg. per gm. dry weight*
Liver, pork	1.2	Tongue, beef	0.4-0.5
Liver, lamb	1.2	Veal	0.5
Liver, veal	0.9	Brain, beef	0.3-0.5
Kidney, pork	0.85-1.0	Heart, pork	0.3
Yeast, brewers	1.0	Heart, beef	0.3
Yeast, bakers	0.5	Dried cereal grasses	0.1-0.15
Pork, loin	0.45-0.6	Skim milk powder	.05-.15
Pork, ham	0.4	Wheat germ	.05-.1

The response of each dog to standard amounts of nicotinic acid was determined before any assays were started and again after two or three assays had been made. The response measured was that obtained from a single dose of food material, all of which was consumed within a period of 24 hours.

Table I summarizes some of our results calculated as milligrams of nicotinic acid per gram of dry food. Our results are somewhat higher than those given by Bandier. It is entirely possible that the response shown by dogs when given liver as a source of nicotinic acid is somewhat greater than that resulting from nicotinic acid alone, but we must not overlook the possibility of incomplete extraction in the chemical procedures. If we assume the daily human requirement for nicotinic acid is 25 mg., then 25 gm. of dry liver or one-quarter of a pound of fresh liver will supply the requirement. About one-half pound of lean meat per day will amply meet the need.

In the assay work it became apparent immediately that only certain foods will produce rapid improvement when given at levels which will be consumed readily by the sick dog. This group includes practically all animal tissues and yeast. Foods like wheat germ and skim milk gave a response only when the supplements were continued over a period of several days. The availability of a reliable chemical method will greatly facilitate the work on the distribution of this vitamin in foods. In gen-

TABLE II

ANTI-BLACK TONGUE ACTIVITY OF VARIOUS PYRIDINE DERIVATIVES

Active	*Inactive*
Nicotinic acid	Pyridine
Nicotinic acid amide	Picolinic acid
Ethyl nicotinate	Isonicotinic acid
Nicotinic acid N methyl amide	Nipecotic acid
Nicotinic acid N diethyl amide	6-Methyl nicotinic acid
β-Picoline	Trigonelline
Nicotinuric acid	1-Methyl nicotinic acid amide chloride
	Quinolinic acid
	β-Aminopyridine

eral we may say that most natural foods will vary from 1 to 100 mg. of nicotinic acid per 100 gm. dry material. Certain crude liver extracts may contain as high as 200 to 300 milligrams per 100 grams. In order to supply amounts of nicotinic acid which have proved effective in the treatment of pellagra, it would be necessary to feed at least 100 grams of the more concentrated sources.

As long as severe pellagra is encountered, I imagine the use of nicotinic acid or related compounds will have to be continued, but when it is used we must remember that there are at least five other members of the B complex which may also be low in the diet of pellagrins. Work both in the field and with dogs on the Goldberger diet indicates a possible deficiency of thiamin and riboflavin.

Dogs grow very well on the Goldberger diet supplemented with thiamin, riboflavin and nicotinic acid, but if the corn in such a diet is replaced by sucrose the dogs will grow for only a short time and then begin to lose weight. The addition of 2 per cent of liver extract renders the diet complete. We are still working on the fractionation of liver extract, but to date we are certain that the liver extract must supply at least three additional factors, vitamin B_6, factor W, and pantothenic acid. If the dogs are deprived of vitamin B_6 they will develop a severe microcytic anemia which responds readily to the administration of 60 micrograms of synthetic vitamin B_6 per day. When the basal is supple-

mented with vitamin B_6 alone there is no growth until pantothenic acid and factor W concentrates are added.

Thus it is important to follow the nicotinic acid therapy with foods rich in other members of the B complex. Nicotinic acid as such is an emergency measure. Our goal should be the modification of the diet so that the people existing on marginal diets would obtain nicotinic acid as well as other essentials from foods. This does not mean that certain foods low in nicotinic acid but relatively rich in other essentials could not be fortified when experimental work has shown the proper means of fortification. The dogmatic statement that foods must not be fortified with nicotinic acid or other synthetic vitamins should not be accepted until all the facts are available.

Let us now turn to the second question: Are other compounds equally as effective as nicotinic? Shortly after we demonstrated the effectiveness of nicotinic acid in the dog, Mr. Madden and I, in conjunction with Strong and Woolley,[47] tested the activity of a number of related pyridine derivatives. The active and inactive compounds are listed in Table II. It was immediately apparent that a rather specific structure is required for anti-black tongue potency. The alpha and gamma isomers of nicotinic acid (picolinic acid and isonicotinic acid) are completely inactive. All the compounds tested in which one of the ring hydrogens had been substituted (by a methyl or a carboxyl group) or in which a methyl group had been added to the ring nitrogen were inactive. The replacement of the carboxyl group of nicotinic acid by a sulfonic acid group or by a cyano group or the removal of the carboxyl entirely (i.e., pyridine) led in each case to inactive compounds.

It appears probable that in addition to the acid and its amide only those compounds possess anti-black tongue potency which are capable of oxidation on hydrolytic conversion to these substances in the body. Evidence for this view is based on the fact that alkyl substituted amide proved to be active as did the ethyl ester. β-picoline which might be expected to be oxidized in the body to nicotinic acid showed a fair degree of activity. Nicotinuric acid was also active, which indicates that the body can hydrolyze this dipeptide. Subbarow, Dann, and Meilman[48] reported in a preliminary note that β-aminopyridine was highly active in the treatment of black tongue. When this compound was tried in our laboratory, we[49] found it to be completely inactive. In a later note Subbarow and Dann[50] confirmed our findings.

It is interesting that there is a close correlation between the results obtained in our work with dogs and those obtained by Dorfman, Koser, and Saunders[51] with the dysentery bacillus. Since they found β-picoline to be completely devoid of growth-promoting activity the organisms evidently cannot oxidize the methyl group while the animal body can bring about this transformation to a limited extent.

Recently there has been considerable interest in the activity of related pyrazine and thiazole compounds. Dr. Bills has prepared both the mono and the 2,3 dicarboxylic acid derivatives of pyrazine, and Spies (personal communication) has found that they show some activity in pellagra. We have also found that they possess about one-tenth the activity of nicotinic acid in the treatment of black tongue. Dr. Schmelkes has sent us thiazole 5 carboxylic and it also shows some biological activity. To date no compound has been found which is more effective than nicotinic acid or the amide.

At this point we should say a word about the toxicity of nicotinic acid. The work of both Chen, Rose and Robbins[52] and of Unna[53] indicates the very low toxicity of nicotinic acid and its derivatives. Sodium nicotinate showed a toxicity in mice and rats only when fed at levels ranging from 4 to 7 grams per kilogram body weight. The amide was found to be somewhat more toxic than the sodium salt. Unna found that prolonged oral administration of 2 grams per kilogram daily of sodium nicotinate to rats, chickens, and dogs over periods up to 2 months failed to produce toxic symptoms. The work of Chen, as well as that in our own laboratory showed that dogs receiving 2 gm. of nicotinic acid per day for several days showed some toxicity. However, Unna suggests that the toxicity may have been due to the acidity of the nicotinic acid since he observed no ill effects with the neutralized compound in even larger doses. In any case there seems to be the same wide range between therapeutic dose and toxic dose for nicotinic acid as for the other vitamins.

In addition to the above results practically all investigators have found that the administration of large amounts of nicotinic acid to human beings is often followed by sensations of heat and tingling of the skin. This feeling is accompanied by flushing and rise in skin temperature. In normal individuals the intravenous injection of 20 mg. nicotinic acid or its salt will cause an increased skin temperature. According to Spies, Bean, and Ashe[32] the chemicals effective in pellagra therapy do

not always produce flushing but those provoking the temperature rise are all effective therapeutic agents. Sebrell and Butler[54] found reactions in some persons on continued treatment with daily doses as low as 30 mg. by mouth. They conclude that the occurrence of these transient reactions should not be allowed to interfere with the therapeutic use of large doses of nicotinic acid.

As soon as the nutritional significance of nicotinic acid was recognized, it was generally assumed that its function in the animal body must be related to coenzymes I and II. However, it was not easy to obtain direct evidence for this relationship. Both coenzymes are very important in carbohydrate metabolism and they are supposed to differ in structure only by one molecule of phosphoric acid, yet they possess remarkable specificity in relation to the dehydrogenases with which they will react. In most cases a substrate together with its dehydrogenase will react with one of the coenzymes but not with the other. The quantitative estimation of the amount of coenzyme present in tissues is based upon this specificity. Of the two factors, coenzyme I or cozymase is most easily estimated.

The most obvious approach to any study on the function of nicotinic acid is therefore the estimation of the coenzyme content of the tissues from animals suffering from nicotinic acid deficiency. Euler and co-workers[55] used this approach on rats but unfortunately a specific nicotinic acid deficiency was not produced in the rats. Very recently Euler and co-workers[56] have presented results to show that tissues from rats on diets low in the B complex tend to be lower in nicotinamide and cozymase than those from normal rats. However, it remains difficult to demonstrate an uncomplicated deficiency of the pyridine nucleotides or any of their precursors in the rat. We have, therefore, used the dog and the pig in all of our studies.

We have limited our work to coenzyme I and the amount in various tissues has been determined by the yeast fermentation method which has been employed by the Euler group. The method is based on the principle that the addition of varying amounts of coenzyme to a washed yeast preparation will produce rates of fermentation which are proportional, within certain limits, to the amount of coenzyme I added. The rate of CO_2 evolution is measured in a Barcroft differential manometer under very carefully controlled conditions. The optimum levels of washed yeast, glucose, magnesium, manganese, hexose diphosphate, and

TABLE III

THE COENZYME I CONTENTS (EXPRESSED IN MICROGRAMS PER GM. OF FRESH WEIGHT) OF VARIOUS TISSUES

Animal	*Liver*	*Kidney cortex*	*Brain gray matter*	*Gastrocnemius muscle*	*Blood*
Guinea pig	523 (4)*	503 (4)	107 (4)	662 (4)	65-89 (4)
Rat	1114 (6)	1077 (6)	353 (6)	782 (6)	84-106 (6)
Chicken	878 (3)	990 (3)	306 (3)	693 (3)	65-105 (15)
Dog	1185 (1)	1060 (1)	——	458 (1)	51-66 (4)
Human	——	——	——	——	20-35 (10)

* Figures in parentheses indicate the number of animals used.

buffer must be determined for each type of yeast used. Under these conditions the cozymase content of animal tissues may be determined by comparison with standard amounts of pure cozymase provided there is no loss of cozymase during the preparation of the animal tissues for analysis.

Dr. Axelrod, working in our laboratory, has found that the following procedure allows practically complete recovery of the cozymase. The animal is sacrificed in the absence of anesthesia and the desired tissue removed immediately, cut into thin slices, and placed on slabs of solid carbon dioxide. The frozen tissue is ground to a fine powder, placed in boiling water, boiled for 2 minutes, and cooled immediately. A suitable aliquot can then be taken for analysis. When blood is analyzed the red cells are separated and placed directly in hot water.

In Table III are given the results obtained with several different tissues taken from guinea pigs, rats, chickens, dogs, and humans. It is apparent that cozymase content of the tissues from different species does not vary greatly in spite of the apparent difference in the nutritional requirements. The values given in this table are somewhat higher than those found in the literature due undoubtedly to the fact that great care was taken to prevent destruction of the cozymase during the process of extraction.

Our first studies on the effect of a nicotinic acid deficiency involved the determination of the cozymase content of the blood of normal dogs

TABLE IV

THE EFFECT OF A NICOTINIC ACID DEFICIENCY UPON THE COENZYME I CONTENT OF DOG TISSUES

Tissue	*Micrograms of coenzyme I per gram of fresh tissue*		
	Dog IV deficient	*Dog V deficient*	*Dog VI normal*
Kidney cortex	1130	1070	1000
Blood*	60	66	61
Liver	714	650	1185
Gastrocnemius muscle	295	427	490

* No significant changes were found in the hematocrit values.

and dogs suffering from black tongue. Throughout our entire work we have been unable to show any decrease in the blood even in very severe cases of black tongue. Similar results have been obtained with blood from pigs suffering from severe nicotinic acid deficiency. It is interesting that values for dogs range from 50 to 60 micrograms of cozymase per cc. of whole blood and for pigs the value is less than 10 micrograms. In both cases all of the cozymase was found in the corpuscles.

Vilter, Vilter, and Spies[45] using the method of Lwoff found a decrease in the cozymase content of the blood of pellagrins and an increase upon the administration of nicotinic acid. Kohn,[46] however, found no difference between the blood of normal and pellagrous patients but did find an increase even in normal patients upon administration of nicotinic acid. Our studies on humans carried out in coöperation with Dr. Edgar Gordon agree with those of Kohn. The value in normal patients was found to be 20-30 micrograms per cc. of blood, which may increase to 50-60 micrograms upon the ingestion of 100 mg. nicotinic acid per day. When the high level is established and the nicotinic acid is no longer supplied, the cozymase gradually decreases to the normal level during a period of about two weeks.

Our next approach was the estimation of cozymase in the tissues taken from the various animals. At first we did not have pure cozymase as a standard, but even in these studies it was evident that both the liver

TABLE V

COZYMASE AND NICOTINIC ACID CONTENT OF BIOLOGICAL MATERIALS

	Cozymase mg. per 100 gm. fresh wt.	*Nicotinic acid calculated from cozymase, mg. per 100 gm. fresh wt.*	*Nicotinic acid found, mg. per 100 gm. fresh wt.*
Liver, beef	70	12.9	26.1
Liver, pork	52	9.6	33.0
Muscle, beef	31	5.7	13.8
Muscle, rat	20*	3.7	——
Blood, human	2-3	.36-.54	.38**
Yeast, brewers	50*	9.2	24.0
Yeast, bakers	25*	4.6	12.0

* Euler — ** Swaminathan

and muscle tissue from deficient dogs contained considerably less than the same tissues from normal dogs. No differences could be detected in the kidney and brain. After the pure cozymase was made available to us by Dr. von Euler, it was possible to calculate the actual amount in the tissues.

The results obtained with one normal dog and two black tongue dogs are given in Table IV. From these figures it is evident that there may be a significant decrease in the liver and muscle depending upon the severity of the symptoms. Similar results have been obtained with pigs. The muscle of the deficient pigs showed a much greater decrease than in the case of the dogs.

It is significant that the brain, kidney, cortex, and blood maintained their normal coenzyme I content under the conditions of our experiments. Perhaps a normal level in these tissues is absolutely essential and a decrease is incompatible with life. In the case of liver and muscle, the vital functions can apparently be maintained although presumably to a greatly reduced degree in the absence of their normal content of coenzyme I. Under more severe conditions changes may also take place in tissues other than the liver and muscle. Whether the decrease in the liver and muscle is sufficient to account for the gross symptoms observed

cannot be answered at this time. However, it is quite probable that the rapid improvement noted both in humans and animals when nicotinic acid is administered is due to the rapid formation of cozymase when the nicotinic acid part of the molecule is made available.

Further evidence for the direct relationship of nicotinic acid found in animal tissues is present as cozymase. We have determined the cozymase content of a number of edible tissues which were also assayed for nicotinic acid potency on dogs. Some of these values are shown in Table V.

If the cozymase is converted to an equivalent amount of nicotinic acid we find that this amount makes up an appreciable amount of the total nicotinic acid. Part of the nicotinic acid must be present as coenzyme II and there is undoubtedly some free nicotinic acid and amide. More definite comparisons can be made when accurate chemical methods for these tissues are available.

In summary we may say that nicotinic acid has a history very similar to that of thiamin and riboflavin. The availability of pure nicotinic acid has not only given us a practical means of combating pellagra but has aided us in understanding some of the metabolic processes which are disturbed during pellagra. Such an understanding will aid in the diagnosis of a nicotinic acid deficiency before the gross symptoms of pellagra develop. We may look for similar results with vitamin B_6 and pantothenic acid as well as other unrecognized factors.

REFERENCES

1. Huber, C. Vorläufige Notiz über einige Derivate des Nicotins, *Liebig's Ann. Chem. u. Pharm.*, 1867, *141:* 271.
2. Funk, C. Studies on beri-beri; chemistry of the vitamine-fraction from yeast and rice-polishings, *J. Physiol.*, 1913, *46:* 173.
3. Suzuki, U., Shimamura, T. and Odake, S. Über Oryzanin, ein Bestandteil der Reiskleie und seine physiologische Bedeutung, *Biochem. Ztschr.*, 1912, *43:* 89.
4. Williams, R. R. The structure of the curative modifications of the hydroxypyridins, *J. Biol. Chem.*, 1917, *29:* 495.
5. Jahns, E. Ueber die Alkaloïde des Bockshornsamens, *Ber. d. deutsch. chem. Gesellsch.*, 1885, *18:* 2518.
6. Ackermann, D. Über das Vorkommen von Trigonellen und Nikotinursäure im Harn nach Verfütterung von Nikotinsäure, *Ztschr. f. Biol.*, 1912, *59:* 17.
7. Szymanska, R. M. and Funk, C. Die Wirkung von einigen Pyridinderivaten auf reisgefütterte Tauben, *Chem. d. Zelle u. Gewebe*, 1926, *13:* 44.
8. Warburg, O. and Christian, W. Co-Fermentproblem, *Biochem. Ztschr.*, 1934-35, *275:* 464.
9. Kuhn, R. and Vetter, E. Isolierung von Nicotinsäure-amid aus Herzmuskel, *Ber. d. deutsch. chem. Gesellsch.*, 1935, *68:* 2374.
10. von Euler, H., Albers, H. and Schlenk, F. Über die Co-Zymase, *Ztschr. f. physiol. Chem.*, 1935, *237:* 1.
11. von Euler, H. and Malmberg, M. Aktivatoren des Kohlenhydratabbaues als

wasserlösliche Nahrungskomponenten, *Biochem. Ztschr.*, 1936, *284:* 455.

12. Funk, C. and Funk, I. C. The value of pyridine derivatives in nutrition, *J. Biol. Chem.*, 1937, *119:* xxxv.
13. Frost, D. V. and Elvehjem, C. A. Further studies on factor W, *J. Biol. Chem.*, 1937, *121:* 255.
14. Sebrell, W. H. Table showing pellagra-preventive value of various foods, *Pub. Health Rep.*, 1934, *49:* 754.
15. Goldberger, J. and Sebrell, W. H. Blacktongue preventive value of Minot's liver extract, *Pub. Health Rep.*, 1930, *45:* 3064.
16. Spies, T. D. Pellagra: improvement while taking so-called "pellagra-producing" diet, *Am. J. M. Sc.*, 1932, *184:* 837.
17. Smith, D. T. and Ruffin, J. M. Treatment of pellagra with liver extracts, *J. Clin. Investigation*, 1933, *12:* 963.
18. Salmon, W. D. and Guerrant, N. B. Liver extract as a source of vitamins B and G_1, *Science*, 1931, *73:* 243.
19. Elvehjem, C. A. and Koehn, C. J., Jr. Studies on vitamin B_2 (G); non-identity of vitamin B_2 and flavins, *J. Biol. Chem.*, 1935, *108:* 709.
20. Kuhn, R., György, P. and Wagner-Jauregg, T. Eine neue Klasse von Naturfarbstoffen, *Ber. d. deutsch. chem. Gesellsch.*, 1933, *66:* 317; 576; 1034; 1577.
21. Lepkovsky, S. and Jukes, T. H. Vitamin G requirements of the chick, *J. Biol. Chem.*, 1935, *111:* 119.
22. Koehn, C. J., Jr. and Elvehjem, C. A. Studies on vitamin G (B_2) and its relation to canine black tongue, *J. Nutrition*, 1936, *11:* 67.
23. Birch, T. W., György, P. and Harris, L. J. Vitamin B_2 complex, *Biochem. J.*, 1935, *29:* 2830.
24. Dann, W. J. Vitamin G complex, *J. Nutrition*, 1936, *11:* 451.
25. Spies, T. D. *Personal communication.*
26. Fouts, P. J., Lepkovsky, S., Helmer, O. M. and Jukes, T. H. Successful treatment of human pellagra with "filtrate factor," *Proc. Soc. Exper. Biol. & Med.*, 1936-37, *35:* 245.
27. Elvehjem, C. A. *et al.* Isolation and identification of anti-black tongue factor, *J. Biol. Chem.*, 1938, *123:* 137.
28. Street, H. R. and Cowgill, G. B. Cure of canine blacktongue with nicotinic acid, *Proc. Soc. Exper. Biol. & Med.*, 1937-38, *37:* 547.
29. Dann, W. J. Nicotinic acid and vitamin B_2, *Science*, 1937, *86:* 616.
30. Sebrell, W. H. *et al.* Nicotinic acid in prevention of blacktongue in dogs, *J. Nutrition*, 1938, *16:* 355.
31. Mickelsen, O., Waisman, H. A. and Elvehjem, C. A. The inactivity of nicotinic acid in chick dermatitis, *J. Biol. Chem.*, 1938, *124:* 313.
32. Spies, T. D., Bean, W. B. and Ashe, W. F. Recent advances in the treatment of pellagra and associated deficiencies, *Ann. Int. Med.*, 1939, *12:* 1830.
33. Cleckley, H. M., Sydenstricker, V. P. and Geeslin, L. E. Nicotinic acid in the treatment of atypical psychotic states associated with malnutrition, *J.A.M.A.*, 1939, *112:* 2107.
34. Katzenellenbogen, I. Nicotinic acid in endemic glossitis, *Lancet*, 1939, *1:* 1260.
35. Landor, J. V. Deficiency of vitamin B_1, *Lancet*, 1939, *1:* 1368.
36. Sebrell, W. H. and Butler, R. E. Riboflavin deficiency in man, *Pub. Health Rep.*, 1938, *53:* 2282.
37. Karrer, P. and Keller, H. Eine kolorimetrische Bestimmung des Nicotinsäureamids, *Helv. chim. Acta*, 1938, *21:* 463.
38. Swaminathan, M. Chemical method for the estimation of nicotinic acid in biological materials, *Indian J. M. Research*, 1938, *26:* 427.
39. Shaw, G. E. and MacDonald, C. A. Colorimetric estimation of nicotinic acid as applied to commercial liver extracts, *Quart. J. Pharm. & Pharmacol.*, 1938, *9:* 380.
40. Bandier, E. and Hald, J. Colorimetric reaction for quantitative estimation of nicotinic acid, *Biochem. J.*, 1939, *33:* 264.
41. Bandier, E. Quantitative estimation of nicotinic acid in biological material, *Biochem. J.*, 1939, *33:* 1130.
42. Knight, B. C. J. G. Nutrition of Staphylococcus aureus; nicotinic acid and vitamin B_1, *Biochem. J.*, 1937, *31:* 731.
43. Mueller, J. H. Nicotinic acid as growth accessory for diphtheria bacillus, *J. Biol. Chem.*, 1937, *120:* 219.

44. Koser, S. A., Dorfman, A. and Saunders, F. Nicotinic acid as essential growth-substance for dysentery bacilli, *Proc. Soc. Exper. Biol. & Med.*, 1938, *38:* 311.
45. Vilter, R. W., Vilter, S. P. and Spies, T. D. Relationship between nicotinic acid and codehydrogenase (cozymase) in the blood of pellagrins and normal persons, *J.A.M.A.*, 1939, *112:* 420.
46. Kohn, H. I. Concentration of enzyme-like substance in the blood following administration of nicotinic acid to normal individuals and pellegrins, *Biochem. J.*, 1938, *32:* 2075.
47. Woolley, D. W., Strong, F. M., Madden, R. J. and Elvehjem, C. A. Anti-black tongue activity of various pyridine derivatives, *J. Biol. Chem.*, 1938, *124:* 715.
48. Subbarow, Y., Dann, V. J. and Meilman, E. The effect of β-aminopyridine in experimental blacktongue, *J. Am. Chem. Soc.*, 1938, *60:* 1510.
49. Strong, F. M., Madden, R. J. and Elvehjem, C. A. The ineffectiveness of β-aminopyridine in blacktongue, *J. Am. Chem. Soc.*, 1938, *60:* 2564.
50. Subbarow, Y. and Dann, W. J. The inactivity of β-aminopyridine in blacktongue, *J. Am. Chem. Soc.*, 1938, *60:* 2565.
51. Dorfman, A., Koser, S. A. and Saunders, F. The activity of certain nicotinic acid derivatives as growth essential for the dysentery bacillus, *J. Am. Chem. Soc.*, 1938, *60:* 2004.
52. Chen, K. K., Rose, C. L. and Robbins, E. B. Toxicity of nicotinic acid, *Proc. Soc. Exper. Biol. & Med.*, 1938, *38:* 241.
53. Unna, K. Studies on the toxicity and pharmacology of nicotinic acid, *J. Pharmacol. & Exper. Therap.*, 1939, *65:* 95.
54. Sebrell, W. H. and Butler, R. E. Reaction to oral administration of nicotinic acid, *J.A.M.A.*, 1938, *111:* 2286.
55. von Euler, H. *et al.* Zur Kenntnis der Cozymase-Bilanz im Rattenkörper, *Ark. f. Kemi., Mineral. o. Geol.*, 1938, *12A*, No. 25.
56. von Euler, H. *et al.* Nicotinsäureamid und Cozymase in normalen und in avitaminotischen Ratten, *Ztschr. physiol. Chem.*, 1939, *258:* 212.