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I hereby recommend that the thesis prepared under my supervision by Sue Potter Vilter
entitled Studies on the Metabolism of Nicotinic Acid

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Approved by:

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STUDIES ON THE METABOLISM OF NICOTINIC ACID

A dissertation submitted to the

**Graduate School
of the University of Cincinnati**

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by

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I wish to express my appreciation to
Doctor Albert P. Mathews and to Doctor Tom D. Spies
for the encouragement and advice which they gave
unsparingly during the progress of this research. The
most gracious acknowledgement would describe inadequately
the contribution which their constant interest has made
to its development.

Nicotinic acid is the beta carboxylic acid of pyridine which has frequently been found associated with food concentrates of high vitamin content (1). Many of the early students of accessory nutritional factors examined this substance because of its frequent biological occurrence, but lost interest in it after Funk failed to cure polyneuritis in pigeons (2). The therapeutic effect of other pyridine derivatives upon experimentally produced deficiency diseases was studied also by R. R. Williams (3), but no physiological explanation was found for the natural occurrence of these compounds.

Interest in nicotinic acid was slow to revive even after vonEuler and Warburg confirmed the presence of nicotinic acid amide in yeast cozymase and in the coferment from blood cells (4, 5). Chemical and biophysical studies of these enzymes had been made, but their relation to human pathology was unsuspected.

In 1938, Elvejhem et al. reported that nicotinic acid amide was present in normal liver and that it was this therapeutic agent in liver which cured the canine blacktongue which was associated with vitamin B complex deficiency (6). This disease was believed to be the analogue of human pellagra. The basis of this analogy was partially established when Spies, Cooper and Blankenhorn published the results of their spectacular cure of 16 cases of human pellagra with nicotinic acid (7). Their work has been verified in all countries, and they have treated 1000 or more cases since that time.

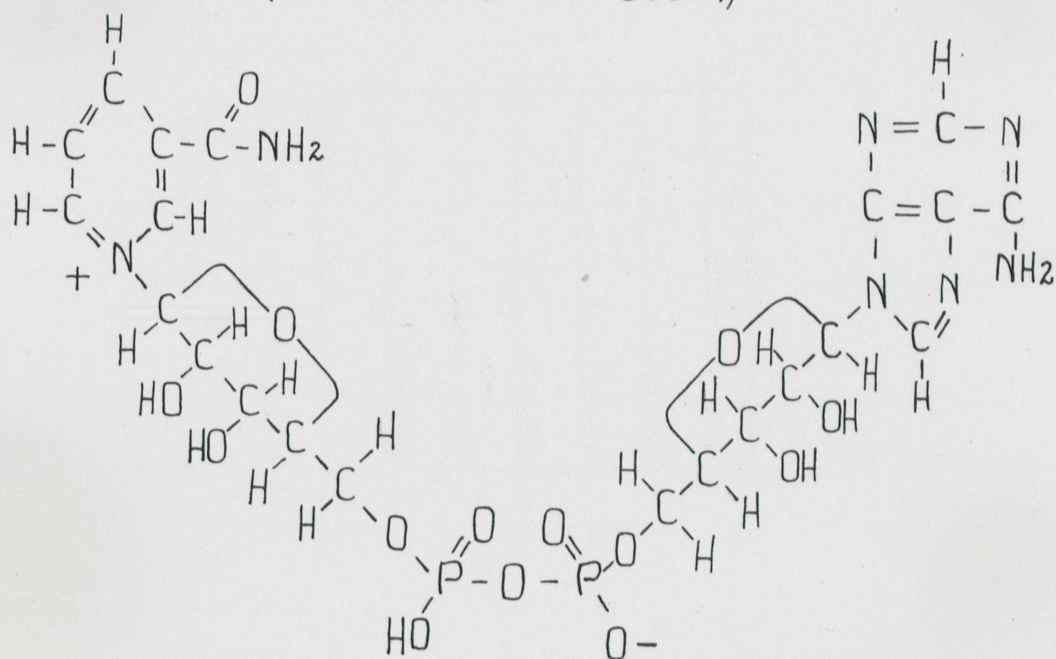
Human pellagra is a general disease, the symptoms of which are most striking in the skin, the central nervous system, and the

mucous membranes of the alimentary and genito-urinary tracts. In a severe case, with all characteristic manifestations, there are dermal lesions of the hands, arms, ankles, the neck, and sometimes of the face. There is severe alimentary tract disturbance. The tongue is reddened, swollen and sometimes ulcerated, the stomach burns, the bowels cramp, and there is intractable diarrhea. The mental symptoms vary greatly. The patients say they are forgetful, confused and depressed. Often, too, they are incoherent and manifest paranoid or manic tendencies.

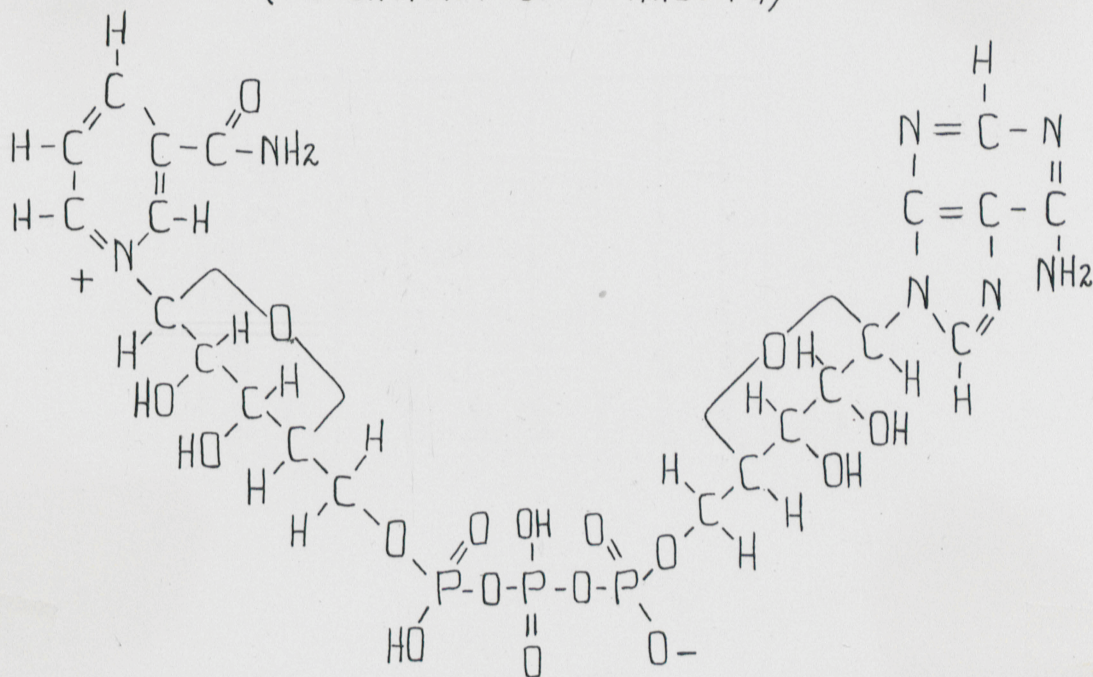
Treatment of pellagrins with nicotinic acid brings rapid relief. The improvement of the alimentary tract is most dramatic. Ptyalism decreases within 4 -- 8 hours, and the tongue begins to heal in 12 hours. The mind of the patient clears and gradually, the dermal lesions heal. In uncomplicated cases the greatest improvement is noted in the first 60 hours after intensive therapy is begun.

It is now believed by most investigators that this new drug is a vitamin which functions in a vital role with a large group of oxidation-reduction enzyme-catalysts. Some of these enzymes are well known in plant life, and their importance to animal physiology is becoming apparent now that the same substances are found in mammalian tissue. Such is the case with the codehydrogenases, cozymase (vonEuler) and coferment (Warburg). Nicotinic acid amide is present in both of these coenzymes, as is seen in the following formulae:

PROVISIONAL FORMULA OF COENZYME I
(COZYMASE OF VON EULER)



PROVISIONAL FORMULA OF COENHYDROGENASE II
(COFERMENT OF WARBURG)



It is believed that the nicotinic acid amide takes on and gives off hydrogen readily between atoms one and six, provided the pyridine N has a quaternary linkage (8). From the formulae above, it is readily seen that these two coenzymes are very similar. Their activity is confined, however, to certain specific substrates; cozymase, CO I, functions when hexose monophosphate is the substrate, while coferment, CO II, is a specific hydrogen carrier when either glucose or hexose diphosphate is the substrate (9). It is possible, however, that in some chemical reactions, the coenzymes act interchangeably.

The following papers deal with the presence of nicotinic acid and certain of its derivatives in body fluids in health and disease, with the physiological mechanism of its use in therapeutics, and with the extent of its absorption, utilization and excretion.

In the first paper ("A Method for the Determination of Nicotinic Acid, Nicotinamide, and Possibly Other Pyridine-Like Substances in Human Urine") a colorimetric method for the determination of nicotinic acid derivatives in urine is described. It is shown that these pyridine compounds are normal excretory products and that the quantity excreted by pellagrins per day is decreased abnormally. The determination of the nicotinic acid content of blood did not prove to be practical by this method. At the present time, however, it is possible to estimate the amount of nicotinic acid in the blood biologically, using the growth properties of dysentery bacilli as an index (10).

In the second paper ("Further Observations on the Effect of

2, 6-Dimethyl Dinicotinic Acid and Dinicotinic Acid on Pellagrins in Relapse and on Normal Persons") it is shown that other nicotinic acid derivatives such as dinicotinic acid and 2, 6-dimethyl dinicotinic acid are antipellagric and increase to some extent the excretion of nicotinic acid derivatives in the urine. The third and fourth publications ("Relationship Between Nicotinic Acid and a Codehydrogenase (Cozymase) in Blood of Pellagrins and Normal Persons" and "Determination of the Codehydrogenases I and II (Cozymase) in the Blood of Diabetics in Severe Acidosis") deal with a new biological method for the estimation of the codehydrogenases I and II in blood and urine. The chemical and technical details of these methods have been devised independently by the author of this paper. The clinical application has been conceived and directed by the coauthors, whose names appear on the above mentioned publications. These publications constitute the major portion of this thesis, and should be referred to for details of method, results and conclusions. Inasmuch as data, as yet unpublished, has been accumulated, which illustrates the relationship of these dehydrogenase enzyme systems to health and disease, a brief account, including tables and illustrations, will be added as a supplement.

**A METHOD FOR THE DETERMINATION OF NICOTINIC
ACID, NICOTINAMIDE, AND POSSIBLY OTHER
PYRIDINE-LIKE SUBSTANCES IN
HUMAN URINE**

By S. P. VILTER, TOM D. SPIES, AND A. P. MATHEWS

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pyridiniumammonium hydrate also have been reported in normal human urine, it is possible that the color developed in normal urine may be due in part to compounds other than nicotinic acid or its methylated betaine, trigonellin. But all sub-

stances producing color by this reaction have been absent from the pellagrin urine thus far examined.

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of the method of estimation of nicotinic acid in the urine of pellagrins and a method for its quantitative estimation. The method of estimation of nicotinic acid in the urine of pellagrins is based on the fact that the urine of pellagrins contains a large amount of nicotinic acid which is converted into a colorless compound when treated with a solution of sodium hydroxide. This compound is then treated with a solution of sodium hydroxide and the colorless compound is converted into a colored compound which is then estimated by the method of estimation of nicotinic acid in the urine of pellagrins.

[Reprinted from the Journal of the American Chemical Society, 60, 731 (1938).]

THE ABSENCE OF NICOTINIC ACID IN THE URINE
OF PELLAGRINS AND A METHOD FOR ITS
QUANTITATIVE ESTIMATION

The method will soon be published in full. It requires only 3 cc. of decolorized urine. It is

Central Society for Chemical Research, in Chicago, by Spies, Cooper and Blankenhorn, made it highly desirable to know whether these substances were present in the urine of normal persons and were lacking in pellagrins. Since no method was available for this purpose, one was devised by one of us (Mrs. S. P. Vilter, Horton-Hallowell Fellow from Wellesley College, 1937-1938).

By means of it we have found in the urine of normal persons eating an average, well-balanced diet, a small amount of nicotinic acid, or a substance with a similar color reaction; but not in the urine of normal persons on a diet free from nicotinic acid; and not in persons having subclinical pellagra, or pellagra in relapse. When such negatively-reacting persons are given sufficient nicotinic acid or its amide by mouth, the urine then gives the nicotinic acid reaction.

These results confirm the curative action of nicotinic acid in pellagra, and show clearly that pellagrins are deficient in this substance in the urine. The method aids in the detection of a pre-pellagrous state and makes possible an earlier diagnosis of the condition than has hitherto been possible.

require only 3 cc. of decolorized urine. It is can be produced after the action of 2,4-dinitrochlorobenzene on nicotine, nicotinic acid and nicotinic amide. Essentially the reaction consists of the reaction of 2,4-dinitrochlorobenzene with the tertiary nitrogen of the pyridine ring, and the subsequent decomposition of the addition product with sodium hydroxide. These colored substances resemble that obtained from pyridine, which is related to glutaconic aldehyde, an aliphatic compound with conjugated double bonds. The pyridine derivative is a purple substance soluble in alcohol, that of nicotinic acid is purple-red, and the derivative of the amide is burgundy-red. In each case, the intensity of the color developed with the pure compounds is quantitative, as measured within the limits of 0.1 and 1.0 mg. Pyridine reacts readily, but with nicotinic acid and amide it is necessary to evaporate the respective solutions to dryness on the water-bath with the reagent, the reacting substances appearing to fuse. The residues redissolved in alcohol and treated with measured quantities of clear, 0.1% alcoholic sodium hydroxide develop strong but transient colors. Since trigonellin and methyl-

A METHOD FOR THE DETERMINATION OF NICOTINIC ACID, NICOTINAMIDE, AND POSSIBLY OTHER PYRIDINE-LIKE SUBSTANCES IN HUMAN URINE*

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(Received for publication, April 27, 1938)

The discovery of nicotinic acid in rice bran (1) and in yeast (2, 3) directed attention of biochemists to the natural occurrence of this pyridinecarboxylic acid. Funk suggested that it had some relation to vitamin B, but subsequent investigation showed that it made no part of vitamin B₁ or B₂. The discovery by Warburg and associates (4) that it was present as the amide in coenzyme and by von Euler *et al.* (5) that it was a part of cozymase revived interest in it and indicated that it was playing an important part in the respiratory metabolism of both animal and plant cells. Following these clues, Elvehjem and his associates (6) discovered that its administration would cure the disease of black tongue in dogs, and Spies, Cooper, and Blankenhorn (7) reported its favorable action in the treatment of human pellagra, an action confirmed by others (8).

Since there is no evidence that pyridine can be formed in the human body, it is evident that nicotinic acid or its amide must be an important article of human diet and that it is apparently necessary for the normal health of the mucosa of the mouth and intestine, nervous system, and skin, since these all are impaired in pellagra and improved by the ingestion of nicotinic acid or the amide. It must be necessary for the muscles also, and probably for the liver and some other organs, since it is an essential

* A preliminary report was published (*J. Am. Chem. Soc.*, 60, 731 (1938)).

This study was aided by a grant from the Rockefeller Foundation.

† Horton-Hallowell Fellow from Wellesley College (1937-38).

part of cozymase and of the coenzyme (Warburg *et al.*) of these tissues and organs.

These facts made it desirable to discover a method by which its presence in animal tissues, fluids, and secretions could be detected and the amount present determined. The pressing need for the discovery of a method making it possible to detect a prepellagrous condition, before the well marked skin and other changes were so developed as to make diagnosis easy, led to the first efforts being directed toward a method for measuring the amount in the urine both in normals and in subjects with dietary deficiency diseases, such as pellagra. Such a method was found, as announced in a preliminary communication, and showed at once that pellagrins when untreated, and normal people, when on a diet deficient in nicotinic acid, had no substances in the urine which would develop the color reaction of the method. They excreted no nicotinic acid conjugates, at least in a form which would give the reaction described herein.¹

An investigation of the literature revealed a characteristic color reaction for pyridine and certain of its homologues. It was first described by Vongerichten (10), and was developed by Zincke (11, 12), König (13), Reitzenstein (14), Baumgarten (15), and others. The reaction involved the addition of 2,4-dinitrochlorobenzene to the tertiary nitrogen of the pyridine ring. This addition product was decomposed by amines, such as aniline, or by alkali hydroxides, to yield a deeply colored product. Further study by König showed that cyanogen bromide gave a similar addition product with pyridine and that decomposition by aniline or by alkalies yielded similar soluble colored products. The opinions in regard to the identity of these colored substances have been controversial, but the most reliable evidence indicates that the derivatives are open chain compounds with conjugated double bonds, and that they are related to glutaconic dialdehyde (15). It was decided to try this reaction upon nicotinic acid, the β -monocarboxylic acid of pyridine, and upon certain other related compounds such as the true salts and conjugated derivatives, and nicotine itself, since a study of the literature did not reveal any

¹ After this paper had been submitted for publication, practically the same method was published by Karrer and Keller for the determination of nicotinamide in cozymase (9).

application of this reaction to substances other than the pyridine-like bases.

It was soon observed that free nicotinic acid, the sodium salt, and the amide would not give a color with 2,4-dinitrochlorobenzene and alkali at the temperatures 60–70° and in conditions used in previous work with pyridine and other like bases as described by the authors cited. However, it was noticed that in the absence of a solvent, nicotinic acid and the above reagent fused at a low temperature, between 85–100°, depending upon the proportion of each. After this fusion, the addition of an alcoholic solution of alkali to the residue gave a brilliant purple color. Since this color was similar to the purple pyridine derivative, it was thought at first that the color was due to some pyridine formed by the fusion. Subsequent work, however, showed this to be improbable, since cyanogen bromide, which reacted with the pyridine, did not yield a colored derivative of nicotinic acid under any of the conditions which have been tried.² Furthermore, in an attempt to extract the colored compounds with organic solvents, it was observed that although the colored pyridine derivative was easily extracted with isopropyl and other higher alcohols from the alkaline liquid, the purple derivative of nicotinic acid had a greater affinity for the aqueous alkali layer and would exhibit a partition in the two solvents dependent upon the concentration of the alkali present. The greater the concentration of the alkali, the less went into the alcohol. This gave evidence that the colored substance was acidic and that the carboxyl group remained intact during the reaction with 2,4-dinitrochlorobenzene. The two derivatives, *i.e.* from pyridine and from nicotinic acid, were evidently not identical. Hence the color was not due to pyridine formed from nicotinic acid as had at first been supposed.

At present, after a study of the factors necessary to produce this color on a quantitative basis, it is believed that an addition compound similar to that described by Zincke (11) for pyridine is formed when an alcoholic (95 per cent) solution of nicotinic acid and 2,4-dinitrochlorobenzene is evaporated to dryness at 35–105°. The fusion and reaction are complete within 10 minutes

² Since the paper was submitted, the successful use of cyanogen bromide to produce a colored derivative of nicotinic acid has been described by Swaminathan (16).

at 100–105° after the solvent is evaporated. The addition product is yellowish but develops red color on the addition of alkali. When only a slight excess of the reagent, 2,4-dinitrochlorobenzene, is used, and the colored solutions are diluted to a fixed volume with more of the cold alcoholic sodium hydroxide, the depth of color produced is proportional to the quantity of nicotinic acid or amide taken. The amount of light absorption of the color can then be successfully reproduced for different concentrations of nicotinic acid in the range of 0.1 to 0.5 mg. on the Sheard-Sanford photometer,³ and the amount of nicotinic acid or the amide thus determined. In this photometer colored filters, a photoelectric cell, and a sensitive microammeter are used. Once calibrated for the colored substance under investigation this photometer is very convenient. Still smaller amounts could no doubt be determined, but with less accuracy with this instrument.

Nicotinic acid itself is not the only substance giving the color. Other compounds related to it which have been tested are nicotinamide, sodium nicotinate, coramine (diethyl nicotinamide), trigonellin, picolinic acid, α -picoline, and nicotine. The sodium salt of nicotinic acid gives a purple color as the free acid does. The betaine, trigonellin, gives no color; *i.e.*, a negative test. The amide gives a Burgundy red, a yellow-red in dilute solutions; but the substituted amide, coramine, again gives a purple color. Why the substitutions of ethyl groups for the hydrogen atoms of the amide nitrogen should shift the absorption toward the red is not yet understood. Picolinic acid, the α -monocarboxylic acid of pyridine (m.p. 136°, uncorrected), does not give a colored product under the same conditions. However, a color does develop after the acid is heated with the reagent over a free flame.⁴ The odor of pyridine is then apparent. The closely related base, α -picoline, *i.e.* α -methylpyridine, gives a purple color under the same conditions as pyridine (4) or nicotinic acid, so the "hindrance" to the reaction in picolinic acid due to the carboxyl group in the α position is of interest. The picolinic acid-glycine conjugate, kindly supplied us by Mr. Birnbaum, also failed to develop color under the conditions of the nicotinic acid reaction. Nicotine

³ Obtained from the Central Scientific Company of Chicago.

⁴ This fact was communicated to us by Mr. S. M. Birnbaum who is working on the excretion of picolinic acid.

itself, however, gives a purple color. This fact should be useful, since tests for this alkaloid are not numerous.

The reaction is, then, sufficiently selective and delicate to enable one to make a quantitative estimation of very small

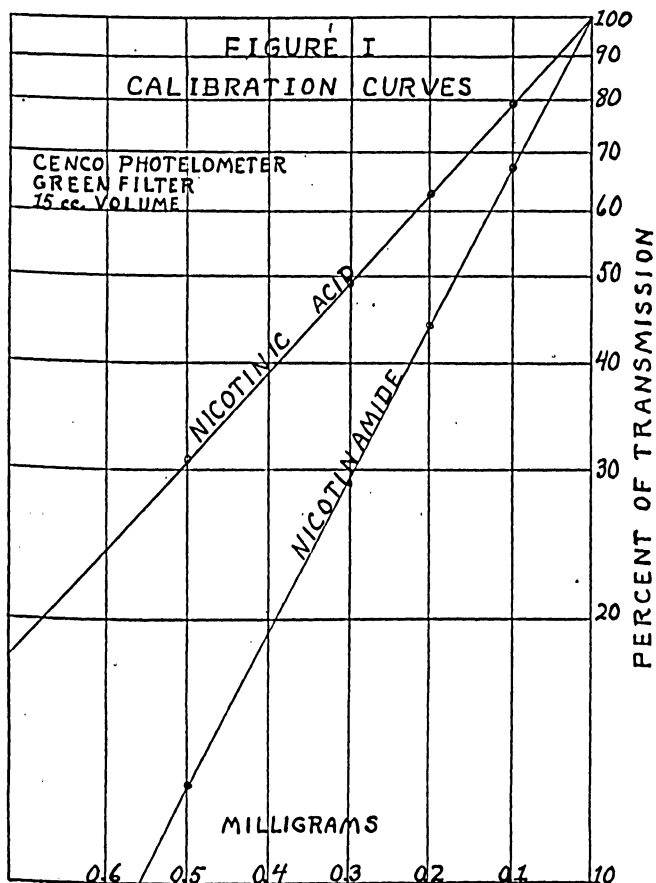


FIG. 1. Calibration chart of nicotinic acid and amide

amounts of nicotinic acid or amide in the absence of other pyridine bases.

The method developed was the following: The photelometer is first calibrated by the use of solutions of the acid or amide of known strength, amounts of solution being taken containing

between 0.1 and 0.5 mg. The calibration curves thus obtained with the green filter of the photometer are given in Fig. 1.

It will be seen from Fig. 1 that for quantities between 0.1 and 0.5 mg. the points when plotted on logarithmic paper with the per cent of transmission of the light, as read from the photometer, as ordinates, fall along straight lines, but that the line of the amide differs from that of the acid.

The standard solutions used to determine the points in Fig. 1 were treated in the following way: 1, 2, 3, and 5 cc. samples of aqueous solutions containing 10 mg. of nicotinic acid in 100 cc., and others containing 10 mg. of nicotinamide in 100 cc., were pipetted into 30 cc. beakers; the solutions were evaporated cautiously at 80–100° or on the water bath and were removed as soon as the solvent had evaporated. To each beaker was then added 1.0 cc. of a 1 per cent solution of 2,4-dinitrochlorobenzene in 95 per cent alcohol. The beakers were allowed to stand for 1 hour or more at room temperature and then evaporated to dryness at 100–105° and heated 10 minutes between these limits of temperature in order to fuse the reagent with the substances to be determined. An excess of the dinitrochlorobenzene must be avoided, as it develops a yellow or orange color by itself when made alkaline. A small excess in the presence of 0.1 to 0.5 mg. of nicotinic acid or nicotinamide is permitted as the color is then too faint to affect the result significantly, but a blank must be run in order to make sure no significant color is produced by the reagent itself in the amounts added.⁵ The 1 cc. of the reagent used by us is sufficient for developing maximum color in 0.1 to 0.5 mg. If more than this be taken, more reagent is needed. The heating should not be higher than 105°, as a brown color may develop at higher temperatures. The amide requires a longer heating to develop maximum color than does the acid. The amide should be heated for 10 minutes between 100–105°, while 5 minutes are enough for the acid. After this fusion, the fusion mixture is cooled to 25°, dissolved in 10 cc. of a clear solution of 0.1 per cent NaOH in 95 per cent alcohol which has been cooled to 10°, and filtered at once.

To test the accuracy of the method when the acid and amide

⁵ Karrer and Keller avoided this difficulty by extracting the excess reagent from the melt with ether. This is an improvement on our method.

are mixed with the organic and inorganic matter of urine, urine was decolorized by filtering through charcoal and then treated with Lloyd's reagent and filtered. Known quantities of the amide or acid were then added to this urine and the amounts determined from the calibration curves made with the pure substances in water. Table I gives the results.

From these results, it is seen that when the procedure is carefully performed to prevent loss of material, and the reaction completed at temperatures consistent with minimum loss and maximum color development, the determination is accurate within 5.0 to 10 per cent. Errors in the determination of aqueous

TABLE I.

Determination of Known Quantities of Nicotinic Acid and Amide

Substance	Amounts determined (average of three or more readings)			
	1 cc.	2 cc.	3 cc.	5 cc.
	mg.	mg.	mg.	mg.
Nicotinic acid				
Solution in water, 5.5 mg. per 100 cc.	0.053	0.114		
Standard solution of acid in treated urine, 10 mg. per 100 cc.	0.109	0.197	0.31	0.509
Nicotinamide				
Solution in water, 3.6 mg. per 100 cc.	0.038	0.072		
Standard solution of nicotinamide in treated urine, 10 mg. per 100 cc.	0.094	0.21	0.293	0.48

solutions of the pure substances may be caused by incomplete reaction and possibly slight losses by vaporization. These, however, can be reduced to a minimum by care.

The next step was to add the known quantities of nicotinic acid or amide to fresh urine, before its decoloration, to see how accurately the amounts could be determined. Table II, containing the results, shows that both the acid and amide may be accurately determined within about 10 per cent when thus added. Furthermore, neither the acid nor the amide was absorbed by the charcoal used for decoloration of the urine. The per cent of error of Table II is calculated from the average of two or more determinations in each case.

Since the color is not permanent but slowly fades after filtration, the influence of the time factor was studied. The results (Table III) show that the maximum color slowly develops in the first 3 to 5 minutes and then fades, but in from 5 to 15 minutes the

TABLE II

Determination of Nicotinic Acid and Nicotinamide Added to Untreated Urine

Untreated urine + nicotinic acid	Conjugate present normally	Nicotinic acid added	Total present	Total determined	Error
cc.	mg.	mg.	mg.	mg.	per cent
1	0.016	0.106	0.122	0.12	-1.6
2	0.032	0.212	0.244	0.244	0.0
3	0.048	0.318	0.366	0.326	-10.9
Untreated urine + nicotinamide		Nicotinamide added			
1	0.016	0.057	0.073	0.079	+8.1
2	0.032	0.114	0.146	0.136	-8.2
3	0.048	0.171	0.219	0.224	+2.2

TABLE III

Stability of Color at Successive Time Intervals

Substance added	Per cent light transmission							
	30 sec.	1 min.	3 min.	4 min.	5 min.	10 min.	15 min.	20 min.
1 cc. nicotinic acid, 0.1 mg. per cc. water	86	82	81.2	80.3	81		82	82.5
1 cc. nicotinamide, 0.1 mg. per cc. water	76	72	70	67	65	67	70	
3 cc. nicotinic acid, 0.106 mg. per cc. urine		51	48	48		49		50
3 cc. nicotinamide, 0.057 mg. per cc. urine		53	49	48	48	49.1	51.2	52

per cent of transmission as read with the filter remains very constant.

Method and Results When Applied to Urine—In the urine of people who have ingested nicotinic acid or amide there might be trigonellin, nicotinic acid, nicotinic acid-glycine conjugate, and nicotinamide. It has been shown that in dog urine the acid,

the glycine conjugate, and trigonellin are present after ingestion of considerable quantities of nicotinic acid. In rabbit urine only the glycine conjugate and free acid are present (17, 18). Of these substances trigonellin, which is known to occur sometimes in human urine, gives no color reaction by our procedure. The acid, if present, gives a purple, the amide a red, and the color of the glycine conjugate has not been determined but probably would be a red. Actually we have found that if not more than 100 mg. of nicotinic acid are ingested, the color given by the urinary constituent is red as with the amide; but if large single doses of 100 to 500 mg. are ingested repeatedly, the color of the urinary constituent for the next few hours after each such dose is the purple color of nicotinic acid or its sodium salts. We interpret this to mean that in the first case either the amide or a conjugate is present. And only when large doses are given does the free acid or a salt appear in the urine.

The method worked out for the urine was as follows: 15 cc. of urine are decolorized by boiling with 0.1 to 0.3 gm. of Darco charcoal (Coleman and Bell). Crude vegetable charcoals do not absorb nicotinic acid (1). The solution when filtered should be clear and colorless. 3 cc. samples are then measured into 30 cc. beakers and are evaporated just to dryness in an oven at 80-100°. When dry, the beakers are removed and 1 cc. of an alcoholic solution of 2,4-dinitrochlorobenzene (1 gm. in 100 cc. of alcohol) is added. 10 mg. of 2,4-dinitrochlorobenzene are sufficient theoretically for development of maximum color with about 5 mg. of both acid and amide. After the samples have stood at room temperature with the reagent for 1 to 3 hours to allow for an intimate mixture, they are again evaporated to dryness and heated 10 minutes at 105°. The temperature must not exceed 105°. 10 minutes of heating at 105° gives the maximum color. Samples which are browned by overheating should be discarded.

The beakers are then cooled to 25°, or lower, and 10 cc. of a clear, cold (10°) solution of 0.1 per cent sodium hydroxide in 95 per cent alcohol are added and the residue stirred from the bottom. The colored solution is then made up just to 15 cc. by addition of about 5 cc. more of cold alcoholic sodium hydroxide. Since these alcoholic solutions are not clear they must be quickly filtered while cold. If for any reason the filtration is slow, it may be

facilitated by suction or centrifugation, but we have employed simply gravity, the solution running readily through the filter paper.

The quantitative reading must be made quickly (within 15 minutes) after the color has developed, although the chilled alcoholate retards the fading.

The colored but clear solution is transferred as soon as filtered to the photometer cell, and with a green filter the per cent of transmission of green light by the solution is read from the photometer scale.

A blank determination of the same quantities of the reagent with alkali alone should be run when fresh solutions are prepared, as too concentrated alkali produces a decided yellow color with any excess of the 2,4-dinitrochlorobenzene.

If the urinary color is a red like that caused by the amide, as it usually is, the amount is read from the amide curve and recorded as an equivalent amount of nicotinamide.

If it be a clear purple like that of nicotinic acid, the amount is read from the nicotinic acid curve.

If, however, as may happen after large doses of nicotinic acid have been ingested, the color is a mixture of red and purple, only an approximation or a semiquantitative estimation can at present be obtained with the photometer. This happens, however, only in specially treated patients or when nicotinic acid has been ingested for experimental purposes. Ordinarily the reading is from the amide curve. With the use of special filters the amount of each reacting substance might be estimated separately but with the three filters of the photometer at present available that is not possible.

Results

No red, or purple, color-producing substances have been found to be present in the urine in all the cases of pellagrins in relapse or in the acute disease so far examined. None of these pellagrins has had enough nicotinic acid or its conjugates in the urine to develop color. We also found little or no color to develop in urine tests from normal persons who were maintained for some time on a strictly controlled "pellagra-producing" diet, a diet such as is usually eaten by pellagrins. The constituents of such a diet are

described later. However, the urine from a person suffering from inanition and dehydration, which came to our attention, showed a weak but positive reaction for nicotinamide, although her mouth was sore, like that of a pellagrin. The patient died before an accurate diagnosis could be made.

Several hundred urine specimens have been examined by this method both in normal persons after oral and intravenous administration of various amounts of nicotinic acid and its salts, and in disease. The urines from three pellagrins in relapse gave negative tests before treatment, but both they and persons on a pellagra-producing diet (*i.e.*, a limited diet without nicotinic acid-containing food) showed a quick response to nicotinic acid therapy by increased excretion of color-producing substances in the urine; but the excretion reverted to the low level normal for the individual on such a diet within 24 hours of ceasing ingestion of nicotinic acid.

The amount of nicotinic acid or its amide excreted daily by so called normal persons on usual diets has varied considerably, but the value as determined by the method on the urines of students, internes, and patients not ill of pellagra lay between 20 and 50 mg. daily.

After nicotinic acid was given by mouth, the quantity in the urine was much increased, but never was equal to the amount ingested. How much was converted into trigonellin has not been determined.

The attempt is being made to apply the reaction to blood filtrate, spinal fluid, gastric juice, and tissue hydrolysates. The results are not yet satisfactory, for although it is possible to see a deepening in the color developed in samples of the blood protein-free filtrate taken after nicotinic acid therapy as contrasted with the pretreatment blood, the color is masked by an intense yellow and is different from that of any of the pure substances so far examined.

We have also tested in a preliminary way the alcohol-water (70 per cent) extracts of foodstuffs, but only successfully when the extract was sufficiently light in color. A qualitative test on active liver concentrates, *i.e.* liver concentrates curative for pellagra and pernicious anemia, was positive, but not quantitatively satisfactory because of the natural color of the material.

In the case of dried brewers' yeast, hydrolyzed and unhydrolyzed samples gave small positive qualitative tests. Similar extractives of hydrolyzed and unhydrolyzed foodstuffs used as a pellagra-producing diet gave negative tests. Extractives of hominy grits, corn-meal, white flour, sweet potato, cabbage, spinach, pork fat, and sugar, foods constituting such a diet, all were negative. While these results are interesting as showing an important difference between the above foodstuffs and certain natural foods which contain pellagra-preventive substances, such as liver and yeast, the work is incomplete and the methods must be improved. Some of the disturbing pigments can no doubt be removed by proper solvents.

DISCUSSION

Until the present work was carried out there have been only two pyridine compounds known to occur in normal urine, namely, trigonellin and methyl pyridinium hydroxide. These substances already have the valences of the nitrogen saturated and this seems to prevent any addition product, such as is necessary to the first step of this reaction. For this reason, and because trigonellin has been found on trial not to give the reaction, we believe that neither of these substances will develop color under the conditions of this reaction, although we have not tested the N-methyl pyridinium hydroxide. Hence, a positive test, such as we have obtained, is fairly certain evidence of the presence of nicotinic acid, nicotinamide, certain of its salts, or, possibly, of its conjugated derivative, in normal human urine. Thus a third pyridine compound, nicotinic acid, is indicated as a constituent of normal human urine.

We conclude, after the examination of many specimens, that a failure of the urine to yield a positive reaction by this test indicates a serious deficiency of nicotinic acid or amide in the individual's diet. Hence this may prove to be a diagnostic sign of a prepellagrous condition, of value in those early cases in which the skin and mouth lesions are not yet well developed.

SUMMARY

1. A color reaction has been described for the detection of nicotinic acid, nicotinamide, substituted amides of nicotinic acid, and certain salts of the acid in urine and other fluids.

2. Colors are developed with nicotinic acid, nicotinamide, sodium nicotinate, and diethyl nicotinamide (coramine); no color is developed by trigonellin and picolinic acid.

3. By the application of this method to human urine it has been found that individuals on a normal diversified diet excrete daily color-producing substances equivalent to 20 to 50 mg. of nicotinic acid or its conjugates. We believe these substances to be nicotinic acid conjugates.

4. Pellagrins in relapse, or in the first acute disease, or normal individuals on a diet such as pellagrins usually consume, excrete little if any color-producing nicotinic acid derivatives.

5. Extracts of various foods, such as liver, yeast, and some others known to be pellagra-preventive foods, are shown to contain substances giving this nicotinic acid reaction, but the method does not yet permit a quantitative determination owing to the presence of other disturbing pigments.

6. The findings suggest that this method is useful as a confirmatory test of the pellagrous and prepellagrous states and for studying the excretion of nicotinic acid and other closely related substances which give colored products *in vitro* with 2,4-dinitrochlorobenzene, when alkalinized.

7. Since nicotinic acid or the amide appears to be one of the vitamins, the method enables one to estimate the amount of this vitamin in urine.

8. Work is being continued on the estimation of the quantity of nicotinic acid and amide in different tissues and foods.

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Further Observations on the Effect
of 2,6-Dimethyl Dinicotinic Acid and
Dinicotinic Acid on Pellagrins in
Relapse and on Normal Persons

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FURTHER OBSERVATIONS ON THE EFFECT OF 2,6-DIMETHYL DINICOTINIC ACID AND DINICOTINIC ACID ON PELLAGRINS IN RELAPSE AND ON NORMAL PERSONS*

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In a recent note¹ it was reported that dimethyl dinicotinic acid (2,6-dimethylpyridine-3,5-dicarboxylic acid) and dinicotinic acid (3,5-pyridine dicarboxylic acid) had a temporary beneficial effect upon pellagrins in relapse. The present report is concerned with additional studies on these two substances. An additional preparation[†] of one or the other of these drugs has been given to a total of nine pellagrins in relapse and to two non-pellagrous individuals. These patients received orally from 500 to 1,000 milligrams per day. Each acid, although difficult to dissolve, was completely dissolved in water before administration. The pellagrins in this study

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[†]The 2,6-dimethylpyridine-3,5-dicarboxylic acid was furnished through the courtesy of Dr. Robert Shelton, and the 3,5-pyridine dicarboxylic acid was supplied by Dr. R. T. Major and Dr. Hans Molitor.

were more severely ill than those in the preceding one and showed less response to these substances. Some temporary improvement was noted in five of the cases, the response being characterized by an increased feeling of well-being and a partial decrease in the fiery redness of the tongue. The subsequent administration of nicotinic acid, however, elicited a more complete response.

In view of the clinical improvement, a study was made of the nicotinic amide-like substances in the urine of patients before and after medication with 2,6-dimethyl dinicotinic acid or dinicotinic acid. In preliminary work, it was found that these dicarboxylic acids, *in vitro*, did not react with 2,4-dinitrochlorobenzene to form a colored product in alkali under the conditions required to produce a colored derivative of nicotinic acid. This was not surprising, however, since it had been noted that the carboxyl group decreased the ease of addition to the pyridine nitrogen.² From seven pellagrins in relapse, a series of urine specimens before and after oral medication with 2,6-dimethyl dinicotinic acid was tested in accordance with the method used for nicotinic acid and its amide,² and there was a small, unexplained increase in the color-producing substances excreted in the forty-eight hours following treatment. This was not nearly so marked as after the administration of the same quantity of nicotinic acid, and the excretion occurred more slowly. Similar results were observed after a study of the urine of two hospital patients, not pellagrins, who were maintained on a B-free diet. Following the intravenous administration of 50 to 100 milligrams of the two drugs to four normal persons, there was a similar increase of color-producing substances when the urine was tested with 2,4-dinitrochlorobenzene.

The physiologic response to these drugs was quite different from the response to nicotinic acid. Neither oral nor intravenous administration has been found to produce the flushing reac-

tion and rise in skin temperature which occur after similar doses of nicotinic acid. These vascular reactions seem to be peculiarly characteristic of nicotinic acid and certain of its salts. The intravenous injection of much larger doses of diethyl amide of nicotinic acid ("coramine") or nicotinic acid amide has not been followed by these temperature responses, although the curative properties of the last two substances had been ascertained previously for blacktongue³ and for human pellagra.⁴

These observations show that 2,6-dimethyl dinicotinic acid and dinicotinic acid have only a partial beneficial effect upon pellagrins in relapse, and they should not be used as a substitute for nicotinic acid. Studies of a large series of pellagrins during the past eight years have convinced the authors that nearly all pellagrins suffer from chronic protein deprivation. The authors feel that this protein deficiency is of more importance in the pellagra syndrome than is currently stressed and that perhaps there is a very close interrelationship between protein metabolism and the role of nicotinic acid. At any rate, whenever a specific therapeutic agent such as nicotinic acid is used in the treatment of pellagra, a well-balanced, high caloric diet should be administered simultaneously.

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Relationship Between Nicotinic Acid and a Codehydrogenase (Cozymase)

IN BLOOD OF PELLAGRINS AND NORMAL PERSONS

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CINCINNATI

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RELATIONSHIP BETWEEN NICOTINIC ACID AND A CODEHYDRO- GENASE (COZYMASE)

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Growth factor V for bacilli of the influenza group has recently been identified as a codehydrogenase (cozymase or coferment).¹ The Lwoffs^{1a} have shown that bacilli of this group which require the factor cannot synthesize it from its components, nicotinic acid amide, adenylic acid, ribose and phosphoric acid. Because of the well known importance of nicotinic acid and nicotinic acid amide in the treatment of human pellagra,² it seemed important to investigate the content of factor V in the blood of pellagrins, for the cozymase of von Euler³ and the coferment of Warburg⁴ are the only two known active biologic substances in which

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nicotinic acid amide is found. We have shown that *B. influenzae* can be used to measure accurately the cozymase content of the blood in normal persons and in pellagrins. Furthermore, prolonged treatment of several hundred pellagrins with nicotinic acid has shown that in most cases there is an immediate increase in sense of well-being and vigor and a prompt relief of the symptoms of acute pellagra.⁵ In a rather high percentage of the pellagrins, however, it is necessary to increase the dosage from time to time in order to keep them free from recurrences. In two instances as much as 1,000 mg. of nicotinic acid a day was required.⁶

Manifestations of vitamin B₁ deficiency often appear in the endemic pellagrin who subsists on his usual diet and ingests large amounts of nicotinic acid each day.⁶ This vitamin B₁ deficiency can be prevented, at least for a considerable period of time, by the addition of 10 mg. of crystalline vitamin B₁ to this diet. Likewise, while pellagrins show definite improvement following the administration of nicotinic acid and vitamin B₁, if the patient remains on a deficient diet certain symptoms will again develop in some of the patients. This suggests that even though two essential substances, nicotinic acid and vitamin B₁, are supplied, the diet usually ingested by these patients must be deficient in still another substance or substances.

During the past five years we have attempted to determine the role of riboflavin in human nutrition, but we were not able to demonstrate a clearcut usefulness for this substance as a therapeutic agent in the treatment of deficiency states in human beings. Therefore, four endemic pellagrins were selected for preliminary study in Birmingham, Ala. These pellagrins were maintained on a pellagra-producing diet and were treated by administering from 100 to 400 mg. of synthetic nicotinic acid and 10 mg. of synthetic vitamin B₁ each day as supplements to the diet. Within twenty-four to forty-eight hours after the administration of these substances was begun, great improvement in the patients occurred. They continued to eat the

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General Hospital. Of the pellagrins, H. J. entered in relapse and, after recovery, was maintained on a pellagra-producing diet for two months. (Dr. W. B. Bean and Miss Jean Grant contributed in the dietary control of this patient and in the therapy of the other pellagrins.) F. H. and W. S. entered with mild lesions which healed after rest in bed, although the patients were maintained on a pellagra-producing diet.¹¹

OBSERVATIONS

The blood of normal persons was found to support growth up to dilutions in peptone of 1:12,000 when the procedure outlined was used with loop transfers

TABLE 1.—*Growth of B. Influenzae in Dilutions of Blood **

Control Cases	1:1,000	1:2,000	1:4,000	1:8,000	1:12,000
R. B.	++++	+++	++	+	±
R. W. V.	++++	+++	++	+	±
J. F.	++++	+++	++	+	±
R. G.	++++	+++	+	±	—

* The extent of growth was interpreted as follows: ++++ = very heavy; +++ = heavy; ++ = moderately dense; + = light; ± = just perceptible; — = no growth.

TABLE 2.—*Growth of B. Influenzae in Dilutions of Blood*

Pellagrins	1:1,000	1:2,000	1:4,000	1:8,000	1:12,000
H. J. (before treatment)†.	+++	++	—	—	—
H. J. (after treatment)....	++++	+++	++	+	—
F. H. (before treatment).. <td>++</td> <td>+</td> <td>—</td> <td>—</td> <td>—</td>	++	+	—	—	—
F. H. (after treatment)...	++++	+++	++	±	—
W. S. (before treatment).. <td>+++</td> <td>+</td> <td>±</td> <td>—</td> <td>—</td>	+++	+	±	—	—
W. S. (after treatment)...	+++	+++	++	±	—

† Treatment refers to the administration of nicotinic acid obtained from Merck & Co.

The controls of the culture in autoclaved mediums without added blood were negative in each case. The sterility of the blood that was added was also established.

of a twelve to eighteen hour culture of *B. influenzae* as the inoculum (table 1). In contrast, the blood of the three pellagrins supported growth poorly and only in the lower blood dilutions, i. e. 1:2,000 (table 2). They contained, therefore, much less factor V than normal. The blood of these pellagrins, however, after

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nicotinic acid therapy, supported the growth of *B. influenzae* almost as well as the blood of the normal persons studied. Patient H. J. was maintained sixty days on the pellagra-producing diet before the administration of nicotinic acid. Then, while still on this diet, he was treated for a week with oral doses of nicotinic acid, which varied from 100 to 500 mg. a day. On the seventh day of treatment a sample of his blood permitted a more luxuriant growth of the organism and supported the growth with greater dilutions of blood than previously (tables 1 and 2). The growth of *B. influenzae* in the blood of the two other pellagrins was studied over a period of days. The blood of F. H. supported growth luxuriously twelve hours after a gram of nicotinic acid was given on one day in five oral 200 mg. doses. The blood from the third patient, twelve hours after the same amount of nicotinic acid was given, supported growth well at 1:4,000 dilution and slightly at 1:8,000. Tables 1 and 2 show the amount of growth in the blood of the two groups of persons, but the plus symbols used are arbitrary and minimize the striking differences observed.

CONCLUSIONS

1. The present study shows that the blood of normal persons on well balanced diets supports growth of *B. influenzae* to a much greater extent than the blood of pellagrins on diets deficient in the pellagra-preventive factor. After nicotinic acid therapy, however, the blood of these pellagrins increased to normal growth-promoting activity. This shows that after nicotinic acid therapy cozymase (or coferment) is significantly increased in the blood of these patients.

2. These observations, plus the observation that prolonged treatment of several hundred pellagrins with nicotinic acid has shown that in many instances the dose must be increased from time to time to prevent recurrences of acute symptoms, support the tentative hypothesis that the therapeutic value of nicotinic acid depends on the synthesis of nicotinic acid nucleotide and finally codehydrogenase in the body.

3. These studies show that the administration of riboflavin is beneficial to certain pellagrins in relapse. Since so many of these specific deficiencies occurring from

water-soluble vitamins seem so interrelated, could not the same general biochemical system or systems be involved?

4. From these observations and those previously reported, we continue to recommend large quantities of a liberal and well balanced diet in the treatment of pellagra.

SUPPLEMENTARY REPORT

Since submitting the preceding for publication, our observations have been extended in several ways. The cozymase content of the blood of a pellagrin with mental symptoms was so low that it would not support growth of the influenza bacillus. However, following the administration of 500 mg. of nicotinic acid daily for two days the influenza bacillus grew in the blood dilutions of 1:4,000 and the symptoms of pellagra disappeared promptly. In three cases of diabetes mellitus in severe acidosis the cozymase content was abnormally low but returned to normal following therapy with diet or insulin or following the administration of nicotinic acid. (We do not imply that nicotinic acid should replace insulin and dietary management in the treatment of diabetes mellitus.) At the suggestion of Dr. John W. Spies, determinations of the cozymase content of the blood of two patients with chronic lymphatic leukemia, three patients with malignant tumors and one patient with Hodgkin's disease were made. The blood from the two patients with leukemia, both of whom had been given roentgen therapy, did not support the growth of the influenza bacillus beyond the 1:1,000 dilution, whereas the blood from the patients with malignant tumors and from the patient with Hodgkin's disease supported growth of these bacilli better than the average. The blood from the patients with leukemia still would not support the growth of the influenza bacillus following the administration of 500 mg. of nicotinic acid daily for two days. The patients with malignant tumors and the normal persons selected for controlled study all appeared in good nutritional state. In contrast to the patients with leukemia, a patient with pernicious anemia in relapse showed normal cozymase concentration in the blood as evidenced by visible growth of the influenza bacillus in dilutions of 1:8,000.

The presence of the V growth factor for *B. influenzae* was also demonstrated in the normal urine studied. "Sterile" normal urine supports growth of the organism in dilutions of 1:10, 1:100 and 1:1,000 in the same autoclaved peptone-blood broth.

*Determination of the Codehydrogenases
I and II (Cozymase) in the Blood of
Diabetics in Severe Acidosis*

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DETERMINATION OF THE CODEHYDROGENASES I AND II (COZYMASE) IN THE BLOOD OF DIABETICS IN SEVERE ACIDOSIS.*

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OBSERVATIONS on a large series of pellagrins during the past 8 years have shown that not infrequently clinical pellagra develops in patients with diabetes mellitus, and that pellagrins in acute relapse rapidly become worse when restricted to diets of large amounts of glucose. Pellagrins in relapse excrete in the urine increased amounts of an ether-soluble substance, or substances, giving the color of porphyrin in 25% HCl. This substance disappears promptly from the urine following the administration of nicotinic acid and reappears when nicotinic acid therapy is discontinued.⁶ We used the same method to test the urine from a patient in diabetic coma but with no clinical evidence of pellagra and the results were similar to those observed in pellagrins.⁶ Recent observations by Vilter, Vilter and Spies,^{8a,b} showing that the blood from patients with chronic leukemia and from pellagrins in relapse contains less cozymase and coferment than normal blood and that the content of these coenzymes in the blood of pellagrins can be raised by oral administration of nicotinic acid, led us to apply the same method in the study of the blood from 3 cases of diabetes mellitus. The present report is concerned with this study.

Method. Five cultures of *B. influenzae* and *B. parainfluenzae* were carried on chocolate agar slants, and studied in broth sub-cultures. The broth was prepared with 2% Difco proteose-peptone, 0.6% salt and 2% human blood. This medium, pH 7.6 was heated to 95° C. and filtered; a portion was sterilized by filtration through a Seitz filter and another portion was autoclaved. As was expected, the portion of autoclaved medium was found not to support growth of those members of the influenza group studied, since cozymase, factor V, is destroyed by autoclaving. The unautoclaved medium, in contrast, provided luxuriant growth of these organisms, as has been shown previously by Davis¹ and by Thjötta and Avery.⁷

* This investigation was aided by grants to the University of Cincinnati College of Medicine from the John and Mary R. Markle Foundation, the Busch Fund, and the David May Fund.

The procedure was as follows: 1 cc. of venous blood was added to 19 cc. of sterile distilled water and heated in a water-bath at 85° C. until the color of the solution became brown. The solution was cooled, and after the addition of 1 to 2 drops of sterile N/10 HCl was centrifuged. The supernatant fluid was then added to tubes of autoclaved peptone broth so that the final dilutions of blood added to the prepared medium represented volumes ratios of 1/1000, 1/2000, 1/4000, 1/8000 and 1/12,000. The blood was heated at 85° C. so the proteins could be centrifuged out, and so the labile inhibitory substance in the serum, described by Rivers and Leuschner³ would be partially or totally destroyed.*

This method has been found to be extremely sensitive. In controlled experiments, the addition of a solution of cozymase to autoclaved broth produced heavy growth at a concentration of 1 part per 100 million. Moreover, the sterility of the media and of all the reagents was proved by control tubes set up with each experiment.

Selection of Cases. Three patients admitted to the Cincinnati General Hospital in severe diabetic acidosis (blood carbon dioxide combining power less than 20 vol. %) were selected for study. Case 1 (J. B.), a white boy, 14 years of age, without previous knowledge of diabetes, had been eating large quantities of an adequate and varied diet until he became drowsy the day of entry. Case 2 (E. M.), a negress, had been vomiting for 8 days before admission. Case 3 (E. W.), a negress, had taken no insulin and eaten little food for a week before entry since she had been incapacitated by an acute upper respiratory infection. In none of the cases was there clinical evidence of pellagra nor of severe systemic disease other than diabetes mellitus. Each patient made a satisfactory recovery following routine therapy, consisting of parenteral administration of large quantities of insulin, physiologic saline and a 10% glucose solution. In each case the blood of a member of the hospital staff served as a control for the diabetic blood under investigation.

Observations. As shown in Table 1, the admission blood of the cases suffering from severe diabetic acidosis, when inoculated with loop transfers from a 12-hour culture of *B. influenza*, supported growth of this organism in dilutions of 1/2000 or less. In contrast, the blood of the control series invariably supported growth in dilutions of 1/8000 or more.

Following excellent responses to the routine diabetic therapy described above, determinations of growth factor present in the blood were repeated within an interval of 10 days. The blood of Case 1 was found to support growth of the influenza bacillus in dilutions up to and including 1/8000. Similarly, the blood of Case 3, after the much shorter interval of 3 days, supported growth well in the 1/4000 dilutions.

Case 2 was maintained for 3 days following return of consciousness on a diet of orange juice and egg white. During the last 48 hours

* Dr. H. I. Kohn (Biochem. J., 32, 2075, 1938) has published recently a method similar to ours.

of this period, she was given orally 500 mg. of nicotinic acid, and then her blood was retested for growth factor. Although her blood at the outset, supported minimal influenza growth in the 1/1000 dilution only, following nicotinic acid therapy, *B. influenza* grew well in the 1/8000 blood dilution. Likewise, increased amounts of porphyrin or substances resembling porphyrin were found in her urine before nicotinic acid therapy and disappeared after the drug had been administered.

TABLE 1.—TWENTY-FOUR-HOUR GROWTH OF *B. INFLUENZÆ* IN DILUTIONS OF BLOOD IN AUTOCLAVED PEPTONE-BLOOD BROTH.

Diabetic patients in coma.		Dilutions of blood.					R.B.C.	Nicotinic acid given, mg.	Porphyrin-like substance in urine.
		1/1000.	1/2000.	1/4000.	1/8000.	1/12,000.			
Case 1 (J. B.)	12/12/38	+	±	—	—	—	5.9	..	Not done.
Case 2 (E. M.)	12/23/38	±	—	—	—	—	5.5	..	Positive.
Case 3 (E. W.)	12/31/38	++	±	—	—	—	..	..	Negative.
+18 nicotinic amide in each tube		++	—	—					
+18 nicotinic amide and 1 cozymase in each tube		+++	+++	+++					
+1 cozymase in each tube		+++	+++	+++					
Same diabetic patients after routine treatment and subsequent recovery.									
Case 1 (J. B.)	12/22/38	++++	+++	++	±	—	4.8	..	Not done.
Case 2 (E. M.)	12/26/38	++++	++	+	+	—	4.74	500	Negative.
Case 3 (E. W.)	1/3/39	+++	++	++	—	—	4.7	..	Negative.
Normal cases used as control to viability of culture for each of above cases.									
J. F.	12/12/38	++++	++++	++	+	±			
W. F.	12/23/38	++++	++++	++	±	±			
S. P. V.	12/31/38	++++	++++	++	±	—			
W. F.	12/22/38	++++	++++	++	±	±			
S. P. V.	12/26/38	++++	++++	++	±	±			
R. W. V.	1/3/39	++++	++++	++	+	—			

NOTE.—The extent of growth was interpreted as follows: ++++ = very heavy; +++ = heavy; ++ = moderately dense; + = light; ± = just perceptible; — = no growth. The controls of the culture in autoclaved medium without added blood were negative in each case. The sterility of the blood which was added was also established.

Lwoff and Lwoff² have shown that bacilli of the influenza group require a codehydrogenase for growth, and are unable to synthesize it from its components, nicotinic acid amide, adenylic acid, ribose and phosphoric acid. In a previous paper^{3a} it has been shown that such a factor is decreased in the blood of pellagrins in relapse and is restored by administration of nicotinic acid. Whether a similar restoration could be aided by administering the other components of the enzyme through a mass action like effect is plausible but as yet untried. The evidence advanced above indicates that a similar factor is deficient in the blood of diabetics in severe acidosis. That this factor is cozymase is supported by the following evidence.

A sample of cozymase* was dissolved in distilled water, so that 1 cc. contained 10 γ . Three series of autoclaved tubed media containing 1/1000, 1/2000 and 1/4000 dilutions of admission blood from Case 3 were prepared. Eighteen γ nicotinic acid amide were added to each tube of the first series, the same amount of nicotinic acid amide and 1 γ cozymase to each tube in the second series, and 1 γ cozymase alone to each tube of the third series. The results were conclusive. Those tubes containing cozymase supported luxuriant growth in all dilutions, while those containing nicotinic acid amide alone were equivalent to the untreated diabetic blood, for growth took place only in the 1/1000 dilution. Control tubes showed that the cozymase solution was sterile after filtration and that it alone in concentrations of 1 part in 100,000,000 would support growth in the autoclaved media.

Conclusions. 1. This report shows that the blood of patients suffering from severe diabetic acidosis supports the growth of *B. influenza* to much less extent than does the blood of normal individuals or the same diabetic when regulated or given doses of nicotinic acid.

2. The codehydrogenase I (cozymase) when added to such deficient blood or to autoclaved media without blood supports luxuriant growth of the influenza bacillus, although nicotinic acid amide shows no such growth stimulation.

3. These observations show that not only is codehydrogenase I deficient in the blood of pellagrins in relapse, but also in the blood of diabetics in severe acidosis, and support the hypothesis that the therapeutic effect of nicotinic acid depends upon the synthesis in the body of nicotinic acid nucleotide and finally of a codehydrogenase.

(Note added to the proof by the author). Spies and Koch⁴ have shown that brewers' yeast (Anheuser-Busch, Inc., The Harris Laboratories, Mead Johnson & Co.) and liver extract (Lilly No. 343) both contain cozymase as evidenced by their ability to support growth of *B. influenza* under the conditions of the method described in this study. Corn meal, which is one of the major constituents of the pellagra-producing diet, also supports growth to some extent, but not nearly so well as does the yeast or liver extract which are anti-pellagic materials. Spies and Koch have shown that 20 normal persons, carefully chosen because they were in good health and

* Obtained through the courtesy of Dr. Hans Molitor of Merck & Co.

were known to eat adequate amounts of a well-balanced diet, excreted cozymase in the urine. In these 20 cases dilutions of 1 to 100, and often dilutions of 1 to 1000, supported growth of the *B. influenzae*. Repeated examinations of the urine of a patient (C. K.) with chronic lymphatic leukemia showed that it supported growth of the *B. influenzae* in dilutions of only 1 to 10, and her blood only in dilutions of 1 to 100. The administration of nicotinic acid, 500 mg. daily, by mouth, was followed by a transient increase in cozymase concentration in the blood and urine. Keeping the nicotinic acid intake constant, the addition of 100 gm. of dry brewers' yeast, by mouth, produced a definite increase to where the blood would grow *B. influenzae* in dilutions of 1 to 2000. After keeping the nicotinic acid and yeast therapy constant for several days, an injection of 20 mg. of riboflavin in sterile physiologic solution of sodium chloride was given. This was followed by a spectacular increase in growth supporting activity through the 1 to 12,000 dilutions. Associated with this increase in cozymase content of the blood was a feeling of well-being, and the patient was able to get out of bed and aid in caring for other patients, despite the fact that her red cell count remained around 2,300,000. It is too early to know whether the blood cells are affected following elevation of cozymase in the blood, but persons having as low a concentration of cozymase as this patient might reasonably be expected to improve following a rise of cozymase content in the blood and tissues. On numerous occasions cozymase has been added to various inoculated tubes and invariably it has supported growth of these bacteria, indicating that the variation from the normal controls is the result of a deficiency of cozymase, or some substance which acts similarly, rather than some hypothetical inhibiting substance.

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SUPPLEMENT

Blood Studies on the Content of Coenzymes I and II in Various Diseases

The method whereby the growth properties of the influenza bacillus indicate the amount of CO I and CO II in body fluids was devised originally for use in the study of the pathological physiology of pellagra. Because of the fundamental relationship of these coenzymes to sugar metabolism and respiration, it was thought that variations of the quantity of these coenzymes in blood and urine warranted investigation in other diseases. Though these studies have been in progress only a short time, already they indicate the intimate relationship of enzyme chemistry to human pathology.

Supplementary and confirmatory data have been obtained on forty-five normal persons and thirty-three patients with pellagra and related deficiency diseases. Observations have been made also on ten cases of diabetes mellitus, ten cases of pneumonia, five cases of pernicious anemia, twenty cases of leukemia, eight cases of malignant tumor, three patients with third-degree burns and three normal patients arbitrarily treated with deep X-ray. The results of these studies are shown in Tables I - XI which follow. It should be noted in the tables that the coenzyme (I and II) content of the blood is very low in acute conditions such as diabetic acidosis, pneumonia, certain leukemias and X-ray sickness. These striking abnormalities and the supplementary data on pellagra will be discussed.

Pellagra

Pellagra is the deficiency disease which appears to be most

directly related to the CO I and CO II. The blood of pellagrins in relapse has a coenzyme (I and II) content which is below normal (for normal values, see Table I). Clinically, pellagra responds to treatment with nicotinic acid; concomitantly the codehydrogenase (I and II) content of the blood is increased. This has been demonstrated by a study of the blood codehydrogenases I and II in thirty-three pellagrins both before and after treatment with nicotinic acid, coramine (diethylamide of pyridine), or riboflavin. Twenty-three persons in this group were treated with nicotinic acid. In every case, there was an increase in the codehydrogenase factor of the blood (Table II). Four of these thirty-three cases were treated with coramine and the factors studied similarly. In these cases it was found that the CO I and CO II did not increase to as high a level after coramine therapy as they did after the administration of nicotinic acid (Table III). Neither did the symptoms of these patients respond well to coramine (11). After the coramine study was completed, the same patients were given nicotinic acid. The codehydrogenase (I and II) content of the blood rose again and with sufficient doses of nicotinic acid remained at a normal level, as illustrated in Fig. 2. Six of the thirty-three pellagrins were treated with riboflavin. No increase was noted in the coenzyme (I and II) content of the blood (Table IV). These same cases responded to nicotinic acid therapy and the coenzyme content became normal.

These studies have shown that the factor or factors which are present in normal blood and absent from the blood of pellagrins are of primary importance to cell life. To be able to increase the content

of a coenzyme in human blood by the administration of a synthetic crystalline compound and to observe concomitant clinical improvement is remarkable. Nicotinic acid outside the body has no growth-stimulating effect upon the organism (B. influenzae) which is used in the test for the coenzyme. Therefore, the body must synthesize the active coenzyme or coenzymes from the nicotinic acid administered.

A normal level of coenzymes I and II in the blood of pellagrins treated with nicotinic acid does not infer a return to normal health, however, although all the symptoms of pellagra are improved. This is illustrated by our observation during the past two months that known pellagrins, adequately treated for the prevention of recurrences, may develop clinical beriberi, clinical flavin deficiency or clinical scurvy as a result of their customary inadequate diet (12). Figures 11 - 15 illustrate the lesions around the mouth (cheilosis) which can be relieved by riboflavin and the scorbutic purpura which can and did occur in a patient who was receiving daily doses of nicotinic acid. The purpura vanished after two injections of 250 mgm. of ascorbic acid.

Diabetes mellitus

A study of patients in severe diabetic acidosis has shown that recovery from the comatose condition is accompanied by an increase in the coenzyme (I and II) level in the blood. Since recovery or death in severe acidosis is rapid, it is difficult at present to evaluate nicotinic acid therapy. It is thought that the coenzyme level is elevated more quickly if nicotinic acid is given, along with the routine diabetic therapy. These coenzymes have special significance in such a

condition of disturbed carbohydrate metabolism since their function is related to the normal metabolism of glucose.

Pneumonia

It has been observed in the practical management of pellagrins that persons with acute or chronic infections require more nicotinic acid to maintain a normal level of CO I and CO II (13). This is shown also in the study of ten pneumonia cases before and after crisis induced by serum or sulfapyridine (Table VIII). The coenzyme content of the blood of these patients was low before crisis and rose spontaneously after the temperature had fallen to normal. Another striking instance of the effect of acute infection is seen in Fig. II. The patient described had Type II pneumococcus pneumonia and had received 1,000,000 units of serum, without clinical improvement. Since she was in poor nutritional condition and had a reddened tongue she was given nicotinic acid, 500 mg. a day for five consecutive days. At no time was there a change in the low coenzyme level. It was only after riboflavin (B₂) was given intravenously (5 mg. twice daily) in addition to the nicotinic acid that there was an increase in the coenzyme (I and II) content. Similar observations have been made on a number of cases at the Birmingham Nutrition Clinic by Dr. T. D. Spies and his associates. They have found that less nicotinic acid is required to cure pellagrins in relapse when riboflavin is given in conjunction with the nicotinic acid. The same observation has been made in experimental animals (14), and has chemical significance in view of the recent in vitro experiments of vonEuler (15).

Leukemia

Our experience with twenty cases of leukemia has not been uniform. Although there were several quite different types of leukemia studies, seventeen of the group of twenty had abnormally low coenzyme (I and II) levels. The patients who were available for study over a period of months did not improve after continuous nicotinic acid therapy, nor was there any change in the coenzyme (I and II) content of the blood. Because of this failure to alter the coenzyme level, other agents were tried, but vitamin B₁ (thiamin) and B₂ (riboflavin), both as pure chemicals, also failed. To date, the only substance which has raised the coenzyme level in patients with lymphatic or myelogenous leukemia has been strain G, Anheuser Busch brewer's yeast. * The results of yeast therapy, interpreted in terms of the coenzyme content of the blood, are shown in Fig. 5 - 9. This work, although extended over several months, is unfinished. At present, it is possible to say only that the patient feels subjective improvement when the coenzyme level is high. The pathology of the disease, however, has been unaltered by any of the therapeutic measures used.

X-ray studies

The most recent study, conducted at the Nutrition Clinic in Birmingham, Alabama, has been spectacular in a limited number of cases (four), so these cases will be sketched briefly. Two young patients,

* This yeast is known to contain two and a half times the usual content of yeast coenzymes I and II.

twelve days following hernioplasty, had identical reactions to deep X-ray over the spleen. Before X-ray treatment, the blood of each supported the growth of B. influenzae in a dilution of one part blood to 8000 parts of medium. The porphyrin in the urine was negative. Twelve hours after the radiation, the ability to support growth of the same culture of B. influenzae had dropped to one part to 2000 parts medium, and porphyrin was present in the urine. The porphyrinuria persisted for 48 hours, but the coenzyme content had risen to normal levels in 24 hours. A repetition of the X-ray treatment on the same patients four days later induced a similar reaction, although 500 mgm. of nicotinic acid was given to each patient twelve hours before radiation (Fig. 10). The only difference in the two experiments appears to be in the rapidity with which the codehydrogenases I and II decreased following therapy. Nicotinic acid given before treatment with X-ray seems to have delayed the fall but did not prevent it. It may be, however, that the dosage was inadequate, or that too much time elapsed between the medication and the radiation.

Another case study was made on a subclinical pellagrin who was recovering without specific treatment. Three successive times, at intervals of 48 hours, the coenzyme level of 1/8000 found in his blood was decreased to 1/2000 following X-ray. The X-ray was applied only superficially for 30 minutes to a basal cell carcinoma of the ear. This extraordinary result is illustrated in Fig. 10. The patient did not have X-ray sickness at any time, although he felt "weak" after the treatment.

The results we have obtained on these few cases may be

significant, so the investigation is being pursued. There appears to be a physico-chemical phenomenon caused by the Roentgen ray which induces a state which may be called "chemical pellagra". Other symptoms of X-ray sickness, such as porphyrinuria, gastric distress, and malaise, tend to support this observation. Nicotinic acid has been reported by some workers to alleviate X-ray sickness (16), as has thiamin (17). For these reasons it is probable that other enzyme systems unrelated to coenzymes I and II are affected similarly and that disordered cell respiration is responsible for the symptomatology of X-ray sickness.

Table I

Coenzyme I and II in Normal Blood

<u>Patient</u>	Blood dilution which supported growth of <u>B. influenzae</u>	<u>Patient</u>	Blood dilution which supported growth of <u>B. influenzae</u>
R. W. V.	1/8000	B. H.	1/8000
W. B. G.	1/4000	W. F.	1/12000
G. G.	1/4000	S. P. V.	1/12000
H. K.	1/8000	J. F.	1/12000
S. P. V.	1/12000	B. G.	1/8000
G.	1/8000	W. G.	1/8000
J. F.	1/8000	P. W.	1/8000
S. V.	1/8000	R. V.	1/8000
R. V.	1/8000	R. B.	1/12000
M. K.	1/8000	R. B.	1/8000
C. K.	1/8000	H. C.	1/8000
M. H.	1/12000	N. H.	1/4000
J. G.	1/8000	J. B. B.	1/8000
M. M. H.	1/8000	M. M.	1/8000
J. M.	1/8000	L. C.	1/8000
T. S.	1/8000	S. B.	1/12000
W. A.	1/8000	M. H.	1/8000
M. H.	1/8000	C. H.	1/12000
W. B.	1/8000	O. S.	1/8000
P. T.	1/12000	H. H.	1/8000
R. V.	1/8000	B. C.	1/8000
R. W. V.	1/12000	S. M.	1/4000
S. P. V.	1/8000		

Table II

Coenzyme I and II in the Blood of 23 Pellagrins Treated with
Nicotinic Acid

Blood dilution which supported growth of B. influenzae

Patient	Before Therapy	After Therapy (100-500 mg. / q.d.)
P. S.	1/2000	1/4000
T. E.	1/1000	1/12000
W. G.	1/2000	1/12000
D. D.	1/2000	1/4000
Mrs. J.H.M.	1/100	1/2000
Mrs. J.B.S.	1/1000	1/12000
M. McK.	1/4000	1/8000
F. J.	1/100	1/4000
C.	1/2000	1/4000
H. S.	1/2000	1/4000
H. R.	1/1000	1/12000
H.	1/4000	1/8000
T. L.	1/2000	1/4000
D. W.	1/2000	1/4000
P.	1/1000	1/12000
J. O.	1/2000	1/12000
H. J.	1/2000	1/8000
F. H.	1/2000	1/8000
W. S.	1/4000	1/8000
T. F.	1/1000 (?)	1/4000
P. W.	1/1000	1/4000
E. H.	1/2000	1/8000
R. W.	1/4000	1/12000

Table III

Coenzyme I and II in the Blood of Pellagrins following Administration of Coramine
(Diethylamide of Nicotinic Acid)

Patient	Blood dilution which supported growth of <u>B. influenzae</u>	
	Before Therapy	After Therapy
F. K.	1/1000	1/2000
P. S.	1/1000	1/4000
T. E.	1/1000	1/4000
W. G.	1/1000	1/4000

Table IV

Coenzyme I and II in the Blood of Pellagrins following Administration of Riboflavin

Patient	Blood dilution which supported growth of <u>B. influenzae</u>	
	Before Therapy	After Therapy
P. S.	1/2000	1/2000
A. G.	1/1000	1/2000
J. B. S.	1/1000	1/2000
T. P.	1/2000	1/2000
K.	1/2000	1/4000
E. R.	1/4000	1/2000

Table V

Coenzyme I and II in the Blood of Patients with Leukemia

Patient	Previous treatment	R.B.C.	W.B.C.	Type of leukemia	Blood dilution which supported growth of <i>B. influenzae</i>	
					Before Treatment	After Treatment*
E. H.	X-ray	4.0 m	14,000	Lymphatic	1/2000	
C. K.	(Transfusion (X-ray	4.0 m	21,000	Lymphatic	1/100	1/8000, 1/12000
W. K.	X-ray	5.0 m	8,000	Myelogenous	1/1000	1/4000
W.F.V.	(X-ray (Transfusion	2.3 m	38,000	Lymphatic	1/1000	
C. C.	X-ray	low	100,000	Lymphatic	1/1000	1/4000
S.	Reticulogen	?	?	Lymphatic	1/8000	
H. S.	Vitamins	?	67,000	Lymphatic	1/4000	
S. Y.	---	---	---	Myelogenous	1/2000	
T. E.	(X-ray (Transfusion	1.6 m	300,000	Acute lymphatic	1/100	
W. Z.	---	---	---	Myelogenous	1/2000	
A. M.	---	2.6 m	153,000	Myelogenous	1/2000	
M. M.	None	4.0 m	68,000	Lymphatic	1/1000	
M. H.	---	3.6 m	49,000	Myelogenous	1/100	
H.L.G.	Transfusion	1.3 m	300,000	Lymphatic	1/100	1/4000
W. A.	X-ray	2.3 m	5,900	Lymphatic	1/1000	1/8000
M. P.	Liver extract	4.0 m	165,000	Lymphatic	1/8000	
M. O.	None	2.0 m	330,000	Acute lymphatic	1/100	
A. E.	None	3.5 m	67,000	Eosinophilic	1/1000	1/4000, 1/8000
H.	---	4.0 m	76,000	Lymphatic	1/4000	
R.	---	2.8 m	320,000	Myelogenous	1/1000	

Table VI

Coenzyme I and II in the Blood of Patients in Diabetic Acidosis

Patient	Blood CO ₂ , vol. %	Blood dilution which supported growth of <u>B. influenzae</u>		
		On Admission	After Chemical Control	After various periods on regulated diet
J. B.	12	1/2000		1/8000 -- 10 days
E. M.	20	1/1000	1/1000	1/8000 -- 2 days (500 mg. nicotinic acid)
E. W.	(pH 6.95)	1/2000		1/4000 -- 3 days
P. H.	11.5	1/1000	1/2000 -- 2 days	1/8000 -- 15 days
R. P.	(not in coma)	1/1000	1/1000 -- 2 days	1/12000 -- 17 days
G. V.	21	1/8000	Died of cerebral hemorrhage	
A. C.	55	1/1000	Died in shock	
H.	12	1/4000	Died in shock	
R. H.	(regulated)	1/8000	Discharged	
A. M.	?	1/2000	1/8000 -- 2 days	

Blood dilution which supported growth of B. influenzae

Table VIII

Coenzyme I and II in the Blood of Patients with Pneumonia

Patient	Pneumococcus type	Treatment	Blood dilution which supported growth of <u>B. influenzae</u>	
			Before Crisis	After Crisis
M. D.	II	Sulfapyridine	1/1000	1/2000
P. W.	II	Serum	1/1000	1/4000
M. J.	V	Serum	1/2000	1/4000
M. C.	XXV	Sulfapyridine	1/1000	1/2000
R. W.	VIII	Serum	1/2000	1/4000, 1/8000 12 days later
W. E.	II	Serum	1/2000	1/4000, 1/4000 8 days later
A. G.	II	Serum	See Fig. 5	
N. S.	IX	Serum	1/1000	1/4000
B.	II	Sulfapyridine	1/2000	1/4000
G. F.	V & VI		1/8000	1/8000

Table IX

Coenzyme I and II in the Blood of Patients with Malignancy

Patient	Type and Location of Tumor	Blood dilution which supported growth of <u>B. influenzae</u>	
		1st Determination	Later Determinations
J. R.	Fibrosarcoma	1/4000 (1-5-39)	Died in 48 hours
L. M.	Carcinoma of ovary	1/8000 (1/5/39)	1/4000 (1/30/39)
J. H.	Carcinoma of sigmoid	1/8000 (1/5/39)	1/2000 (1/26/39), 1/4000 (1/30/39)
N. A.	Carcinoma of cervix	1/4000 (1/29/39)	1/2000 (2/9/39)
E. H.	Carcinoma of stomach	1/2000 (1/30/39)	Signed out
H. W.	? Tumor of maxilla	1/2000 (2/10/39)	1/1000 (2/17/39), after four X-ray treatments 1/12000 (2/21/39), after three more X-ray treatments, which produced chills and fever, 1/100 (2/26/39)
M. R.	Carcinoma of cervix	1/4000 (2/10/39)	After insertion of radium needles on 2/14, 1/2000 (2/15/39)
G. M.	Carcinoma of ovary	1/8000 (2/16/39)	After four X-ray treatments 1/100, and after three more X-ray treatments, 1/100

Table X

Coenzyme I and II in the Blood of Patients with Third Degree

Burns

Patient	Blood dilution which supported growth of <u>B. influenzae</u>
E. B.	1/100 (?) died
M. M.	1/100
W. S.	1/1000

Table XI

Coenzyme I and II in the Blood of Patients Treated with X-ray

Patient	Blood dilution which supported growth of <u>B. influenzae</u>	
	Before X-ray	After X-ray
O. B.	1/12000	1/8000
J. R.	1/12000	1/1000 (nausea)
N. B.	1/8000, 4/12 A.M.	1/2000, 4/12 P.M.
	1/8000, 4/14 A.M.	1/1000, 4/14 P.M.
	1/8000, 4/16 P.M.	
	1/12000, 4/17 A.M.	1/2000, 4/18 P.M.
T. B.	1/8000, 4/2	1/2000, 4/3
		1/8000, 4/4
	1/8000, 4/7	
	1/8000, 4/8	
	1/8000, 4/9	1/2000, 4/9
		1/12000, 4/11
A. R.	1/8000, 4/2	1/2000, 4/3
		1/12000, 4/4
	1/8000, 4/5	
	1/12000, 4/7	
	1/12000, 4/8	1/2000, 4/8
		1/12000, 4/10

Figure 1

MAXIMUM BLOOD DILUTIONS OF TWO PELLAGRINS
SUPPORTING GROWTH OF B. INFLUENZAE
- THE PELLAGRINS HAVING RECEIVED SINGLE DOSES OF
NICOTINIC ACID

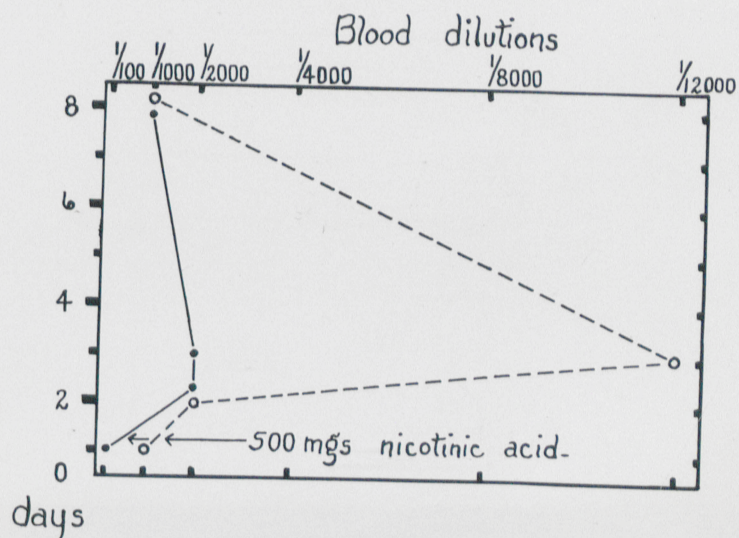


Figure 2

MAXIMUM BLOOD DILUTIONS OF PNEUMONIA PATIENT
SUPPORTING GROWTH OF *B. INFLUENZAE*
AND RESPONSE TO NICOTINIC ACID AND RIBOFLAVIN

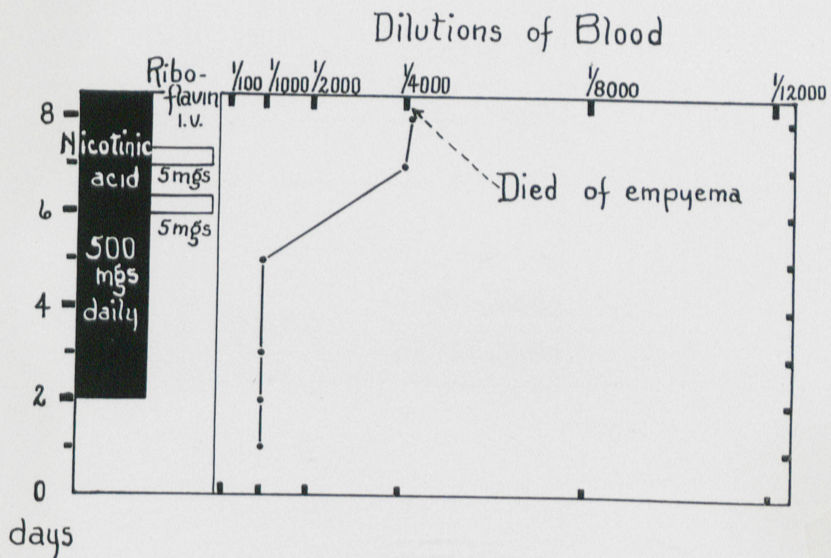


Figure 3

MAXIMUM BLOOD DILUTIONS OF A PELLAGRIN
SUPPORTING GROWTH OF B. INFLUENZAE
AND INFLUENCE THEREUPON OF CORAMINE AND NICOTINIC ACID

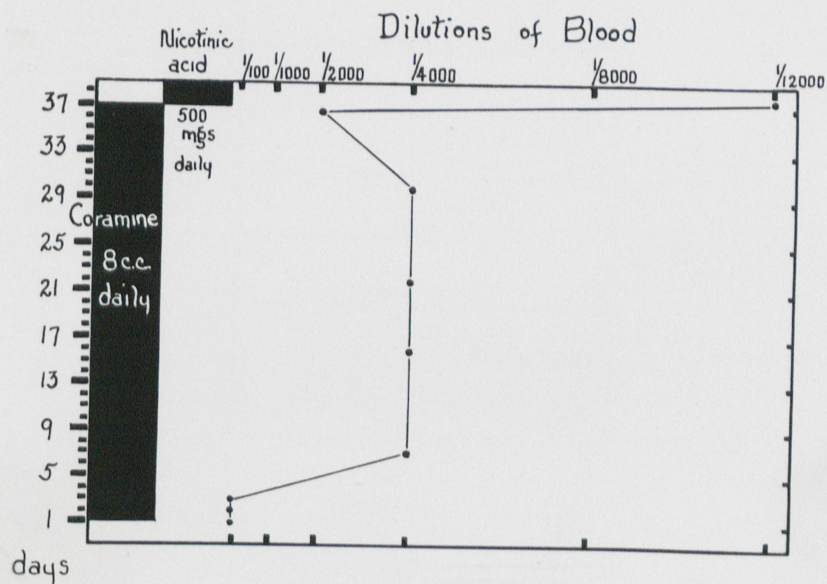


Figure 4

MAXIMUM BLOOD DILUTIONS OF PATIENT ON B-FREE DIET
SHOWING GROWTH OF B. INFLUENZAE

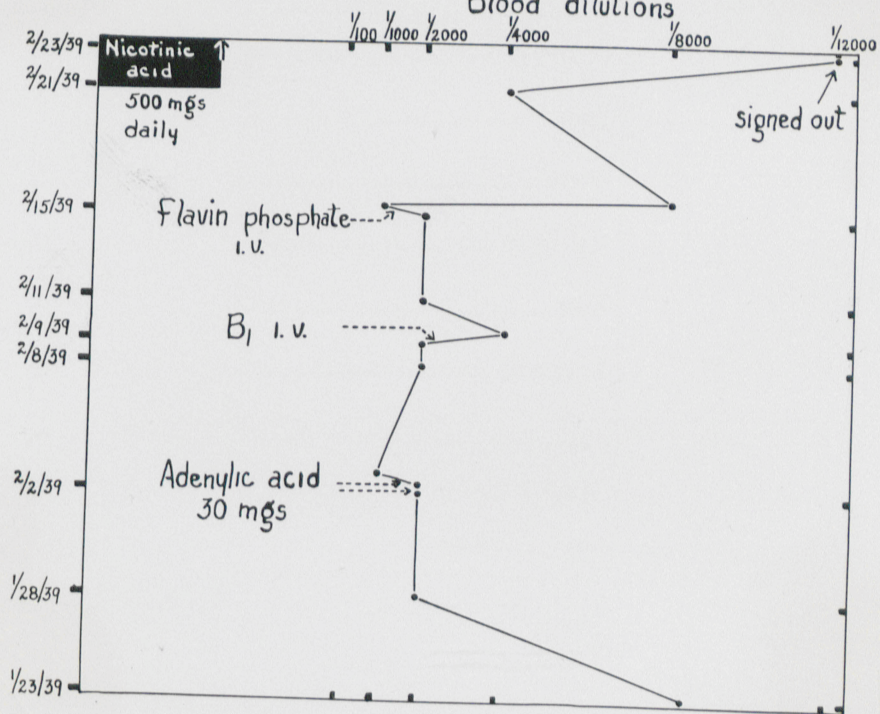


Figure 5

MAXIMUM BLOOD DILUTIONS OF A MYELOGENOUS LEUKEMIC PATIENT
SUPPORTING GROWTH OF B. INFLUENZAE

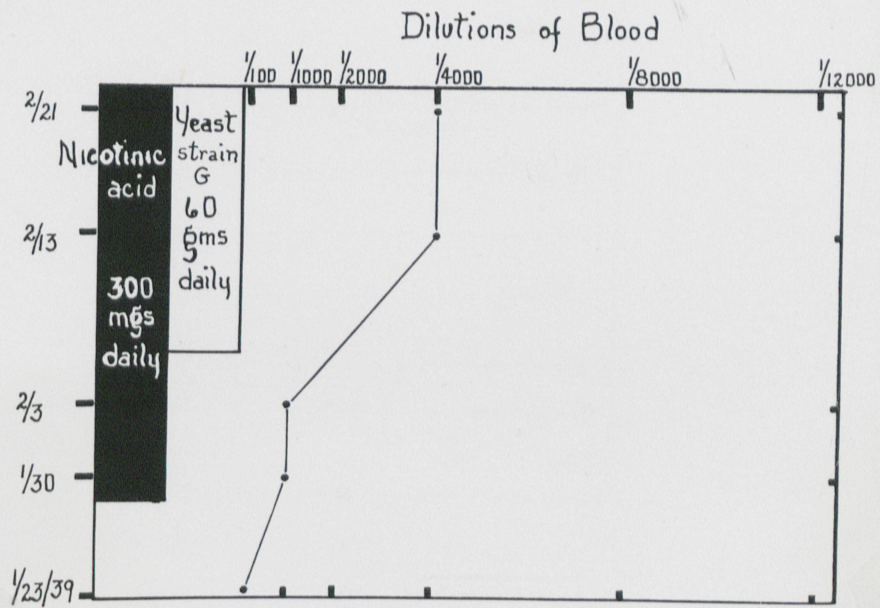


Figure 6

MAXIMUM BLOOD DILUTIONS OF AN EOSINOPHILIC LEUKEMIC PATIENT SUPPORTING GROWTH OF B. INFLUENZAE

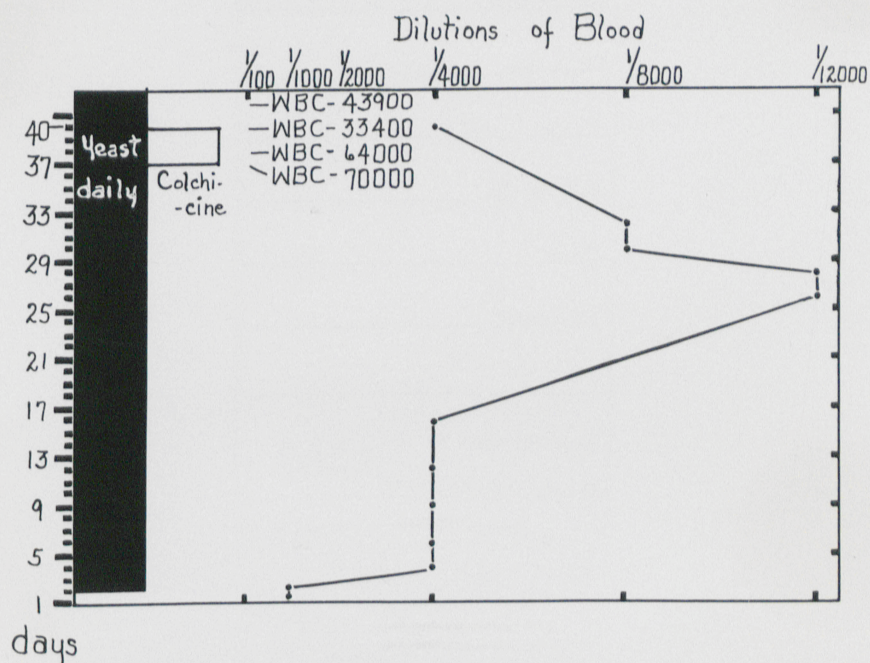


Figure 7

MAXIMUM BLOOD DILUTIONS OF A LYMPHATIC LEUKEMIC PATIENT SUPPORTING GROWTH OF B. INFLUENZAE

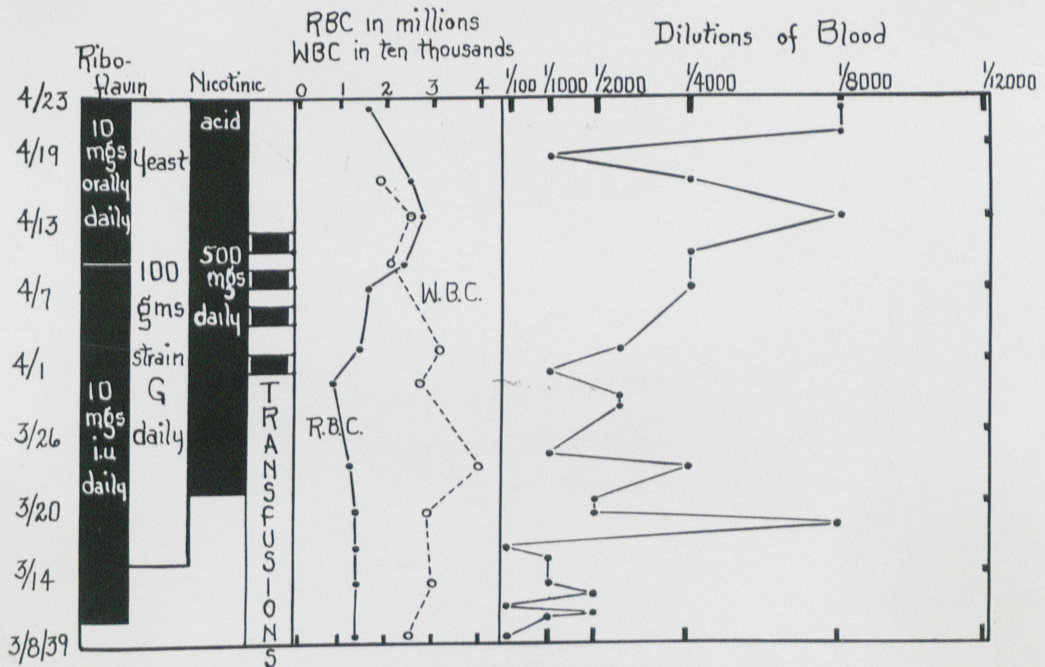


Figure 8

MAXIMUM BLOOD DILUTIONS OF A LYMPHATIC LEUKEMIC PATIENT SUPPORTING GROWTH OF B. INFLUENZAE

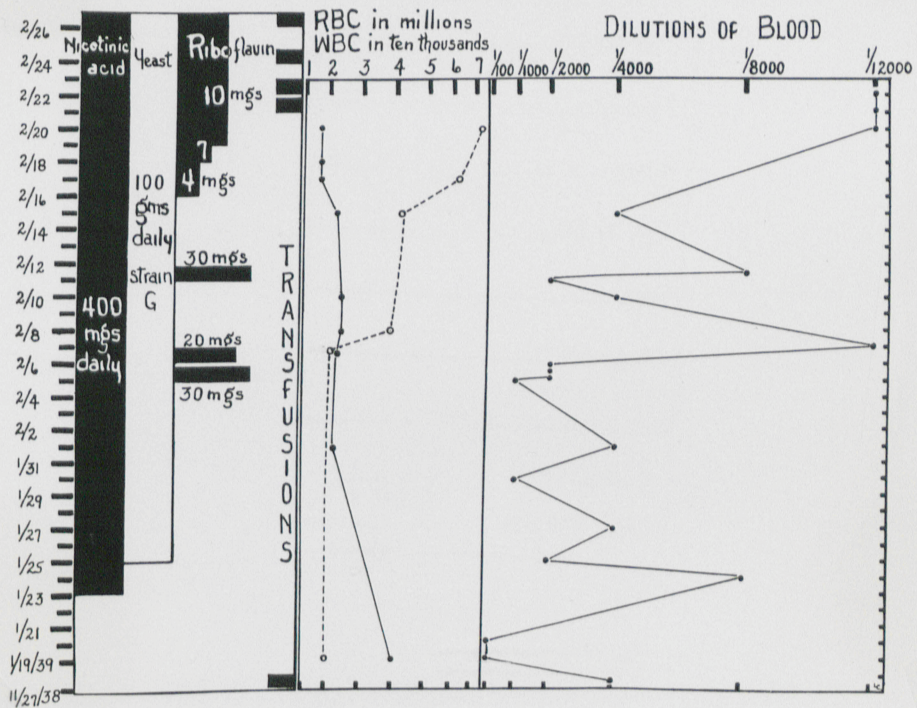


Figure 9

MAXIMUM BLOOD DILUTIONS OF A LYMPHATIC LEUKEMIC PATIENT SUPPORTING GROWTH OF B. INFLUENZAE

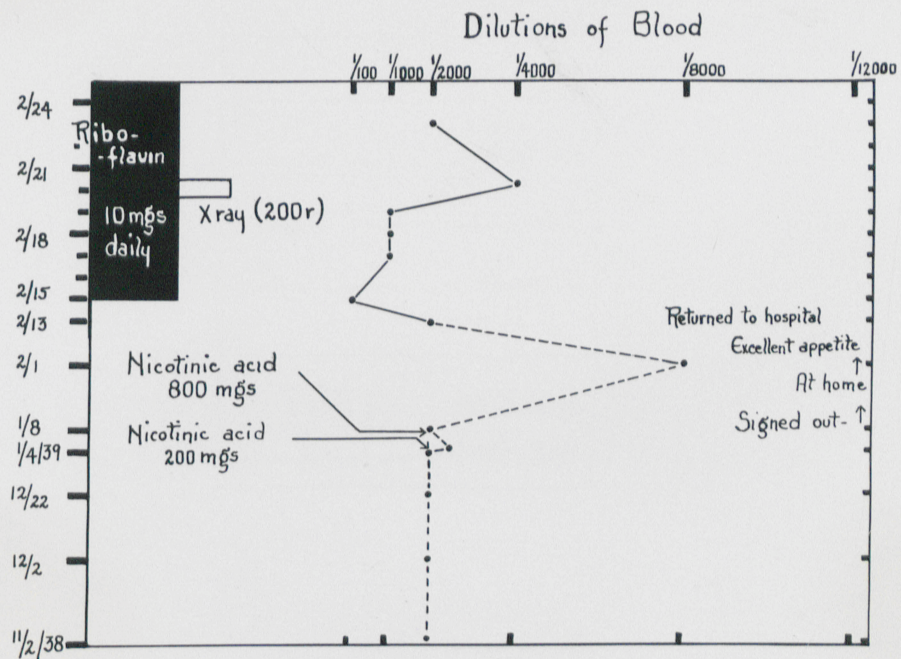


Figure 10

MAXIMUM BLOOD DILUTIONS OF FOUR PATIENTS DURING XRAY TREATMENT SUPPORTING GROWTH OF B. INFLUENZAE

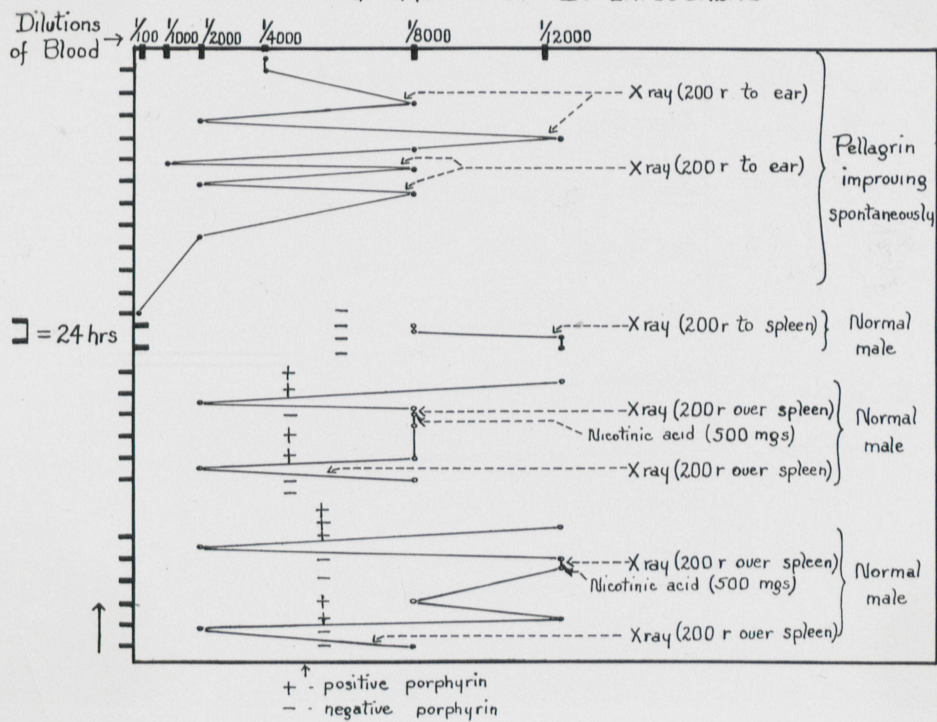


Figure 11



Healing Cheliosis Treated with Riboflavin

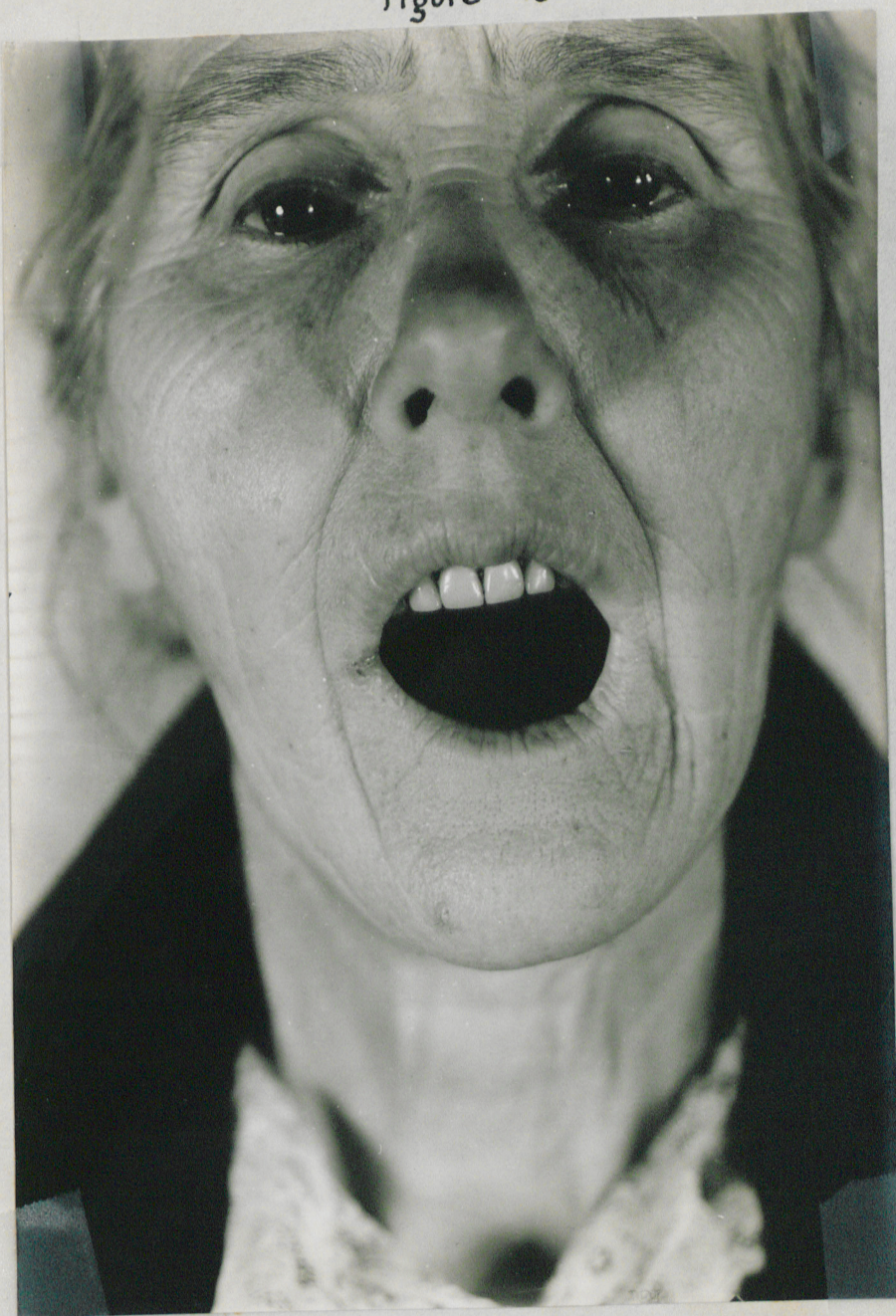
Healing Cheliosis in Same Patient
after eating Riboflavin

Figure 12



Recurring Chielosis in Same Patient
after omitting Riboflavin

Figure 13



Healing of lesion in figure 12 after Riboflavin

Figure 14



Scurvy in a Treated Pellagrin

Figure 15



Pellagra Lesions before Nicotinic Acid Therapy

Figure 16



Same lesions as figure 15, 10 days after
Nicotinic Acid Treatment

Summary and Conclusions

In this investigation, the following points concerning the metabolism of nicotinic acid have been clarified. Many other developments directly related to the problem are still in progress.

1. A chemical method for the determination of nicotinic acid and nicotinic amide in urine has been devised.
2. It has been possible, by virtue of this new method to determine that synthetic nicotinic acid is absorbed rapidly from the alimentary tract in excess of that quantity required by the body, and that it is excreted readily by the kidney.
3. A study of normal persons has shown that the daily excretion of pyridine derivatives in the urine is measurable.
4. In comparison with the urine of normal persons, the excretion of these pyridine derivatives is decreased in the urine of pellagrins before they are treated with nicotinic acid.
5. The chemical relationship of nicotinic acid to the cohydrogenases I and II, cozymase and coferment, which has been suspected for some months, now has been established by means of biological and clinical tests. A method has been devised to measure these coenzymes indirectly, expressing the quantity of these enzymes present in blood in terms of their specific growth-promoting activity for B. influenzae. The study of these coenzymes in the blood of pellagrins before and after nicotinic acid therapy shows indubitably that the intake of nicotinic acid increases the quantity of these coenzymes 10 to 100 times the original level.

6. It has been shown also that cozymase, CO I, and coferment, CO II, are excreted unchanged in normal urine, but that they are rapidly destroyed in urine upon standing. The urine of pellagrins contains from one to ten per cent of the amount of these coenzymes I and II present in normal urine.

7. The codehydrogenase (I and II) content has been studied in the blood of normal persons, in pellagrins, and in persons with certain diseases other than pellagra. The content is decreased in leukemia, in pneumonia, in severe diabetic acidosis, after severe burns, and temporarily in the blood of persons who have received deep X-ray treatment.

8. Nicotinic acid does not raise the content of coenzymes I and II in leukemic blood, although the level can be raised temporarily with strain G, Anheuser Busch brewer's yeast.

These studies are being continued to determine the effect of nicotinic acid, yeast, riboflavin, thiamin and other enzyme precursors upon the dehydrogenase system in normal and abnormal metabolic states.

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