# CCLXVIII. THE CONCENTRATION OF COENZYME-LIKE SUBSTANCE IN BLOOD FOLLOWING THE ADMINISTRATION OF NICOTINIC ACID TO NORMAL INDIVIDUALS AND PELLAGRINS

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THE recent demonstration that nicotinic acid, or its amide, is the vitamin whose deficiency is responsible for canine black tongue and pellagra is of great theoretical interest, for the pyridine ring plays a role of the first importance in cellular respiration. This was demonstrated by Warburg *et al.* [1935] who found their coenzyme (triphosphopyridinenucleotide designated Co II) to comprise one mol. of nicotinic acid amide, three of phosphoric acid, two of (probably) pentose and one of adenine. The nucleotide in the presence of a suitable protein is reduced by the substrate, then reoxidized indirectly by molecular oxygen; this cycle of oxidation occurs in the pyridine ring. Cozymase (diphosphopyridinenucleotide, Co I) differs from Co II only in possessing one mol. less of phosphoric acid, according to Warburg & Christian [1936] and Euler *et al.* [1936]. The mechanism of its cycle of oxidation is the same.

It is almost certain that all active tissues in the human body contain Co I and Co II, and consequently one would expect that variation in amount of dietary nicotinic acid should lead directly to variation in amount of coenzyme. It is the object of this paper to demonstrate that the concentration of coenzyme, or of some closely related substance in the blood, is sensitive to the ingestion of nicotinic acid, both in normal individuals and pellagrins.

The vitamin action of nicotinic acid was first reported for bacteria. Lwoff & Lwoff [1937] proved that Co I or Co II could replace the *V*-factor of the haemophilic bacteria. At about the same time nicotinic acid was shown to be a growth factor for *Staphylococcus* by Knight [1937] for the diphtheria bacillus by Mueller [1937], and recently for the dysentery bacillus by Koser *et al.* [1938].

Frost & Elvehjem [1937] reported that adenylic acid plus nicotinic acid (1 mg. per day of each) completed a basal diet on which rats did not otherwise grow, and Euler *et al.* [1938] obtained a striking response by the addition of 1 mg. per day of Co I (cozymase). However, rat dermatitis (and chick dermatitis) is not due to a nicotinic acid deficiency according to Dann [1937].

Elvehjem *et al.* [1937; 1938] showed that nicotinic acid or its amide is the vitamin whose deficiency is responsible for black tongue in dogs; this has been confirmed by Street & Cowgill [1937] and others, and extended to a pellagra-like condition in swine by Chick *et al.* [1938]. Pellagra, like black tongue, has yielded to nicotinic acid therapy according to Fouts *et al.* [1937], Smith *et al.* [1937], and Spies *et al.* [1938]. Schmidt & Sydenstricker [1938] reported relapses following rapid improvement with nicotinic acid therapy, but neither the diet nor the activity of the patients was controlled, so that at present their results are inconclusive [*vide* also review by Sebrell, 1938].

*Method*

The concentration of coenzyme-like substance, which is confined to the corpuscles, was determined with the aid of *Haemophilus parainfluenzae*, the growth of which under proper experimental conditions is proportional to the coenzyme content of the culture broth.

*Specificity of test*

Lwoff & Lwoff [1937] suggested the use of *H. parainfluenzae* for the bio-assay of coenzyme when they found that:

(1) Either Co I or Co II at concentrations as low as about $2 \times 10^{-9}$ *M* can replace the *V*-factor required for growth by all *parainfluenzae*.

(2) *V*-factor cannot be replaced by adenylic acid (yeast or muscle), nicotinic acid, nicotinamide, diethylamide, *o*-dihydropropylnicotinamide, or the products formed when either coenzyme is kept at 100° and *p*H 8·5 for 20 min.

(3) The reaction Co I $\rightleftharpoons$ Co II can be accomplished by the bacteria when either coenzyme enters the protoplasm, after which its identity appears to remain fixed.

I have found that neither nicotinic acid nor its amide in therapeutically active concentrations, either alone or in the presence of all substances found in the blood extract used for assay, is able to act as *V*-factor, as the following examples show. (A) The addition to blood extract of 1 mg. acid or amide per ml. blood reduced the assay by 4 and 6% respectively. (B) Incubation of whole blood containing 1 mg. acid per ml. at 37·8° for 1 hr. increased the assay by 6%. (C) Incubation of whole blood containing 10 mg. acid per ml. rapidly produced methaemoglobin, haemolysis and loss of *V*-factor.

Products of the mild acid hydrolysis of *V*-factor were found to be inactive, as when blood, diluted 100 times, was boiled for 20 min. in 0·8 *N* trichloroacetic acid containing 0·55 *N* NaOH.

These results are in harmony with those of the Lwoffs; they argue strongly in favour of the unique identity of Co I and Co II with *V*-factor, and prove that only a special and limited group of compounds containing nicotinic acid, or its amide, can be active. However, the terms coenzyme and *V*-factor will not be used interchangeably in the following, and the substance or substances measured in the bio-assay will be referred to as *V*-factor.

*Theory of test*

When broth is inoculated, the optical density of the resulting suspension increases owing to an increase in living and dead bacterial matter, and to changes in the medium itself. The increase in light adsorption is rapid at first, but reaches a maximum at which it tends to remain 24–30 hr. after inoculation, declining slowly thereafter. To measure directly the change in light transmission of a culture it is most convenient to employ the Evelyn photoelectric colorimeter [Evelyn, 1936], which uses test tubes for absorption cells.

Let $T_b$ be the transmission of the blank culture, and
$T_x$ the transmission of the experimental; then
$a$ the % change in absorption is given by (1):

$$a = 100 \left[1 - \frac{T_x}{T_b}\right]. \qquad \ldots\ldots(1)$$

*V*-content is found by determining the amount of blood necessary to produce a value of $a$ equal to that produced by a standard solution.

Let $a_s$ be some set value of $a$ used as a point of reference,

$X$ the concentration (ml./ml.) of blood in the unknown test whose reading is $a_s$,

$S$ the concentration (ml./ml.) of standard $V$-factor in the standard test whose reading is $a_s$, and

$H$ the haematocrit reading (volume of red and white cells expressed *as* $^0/_0$ *blood volume*); then

$V$ the concentration (units/ml.) of $V$-factor in the blood cells, expressed in arbitrary units and multiplied by $10^4$ for convenience, is given by (2):

$$V = \frac{10^4 S}{HX}. \qquad \text{.....(2)}$$

$V$ determined according to (2) will be used as an empirical measure of the concentration of Co I and Co II, bearing in mind, however, that some unknown substance closely related to the coenzymes might give a positive test. No attempt was made to distinguish between the two coenzymes.

### *Procedure of test*

The cultures are prepared in tubes suitable for use in the photoelectric colorimeter, each containing 7 ml. broth. To one series of tubes in duplicate there are added 0·0, 0·1, 0·1, 0·2 and 0·3 ml. blood extract; to a second series there are added similar amounts of standard $V$-factor solution. All tubes are inoculated except the second pair in each series. The tubes are checked in the photometer (using filter 520 $M$) for initial differences in absorption, which are noted and used as corrections. After 24 and 30 hr. of incubation at 37° they are read in the photometer; the maximum value of $a$ is used, and the averages for each pair are plotted as in Fig. 1. Usually the difference between the 24 and 29 hr. readings is small and the duplicates agree fairly well as in the following readings made at 24 hr.:

*Standard V.* 0·1 ml., 2, 1·5; 0·2 ml., 7, 7; 0·3 ml., 11·7, 11.

*Blood extract.* 0·1 ml., 3·3, 4; 0·2 ml., 11·2, 12·2; 0·3 ml., 14, 13.

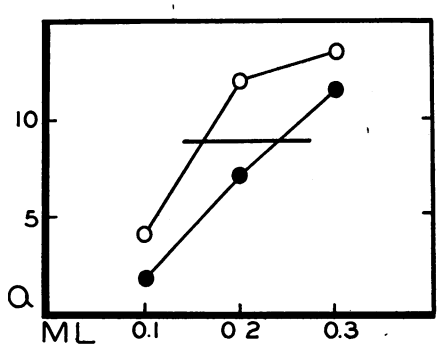


Fig. 1. $a$ as a function of $V$-factor (in ml.). Open circles, $V$-factor from blood extract; solid circles, $V$-factor from yeast extract used as a standard. The bar cutting the two curves at $a=9$ (designated $a_s$) facilitates comparison: 0·16 ml. blood extract is equivalent to 0·245 ml. yeast standard.

The horizontal bar cutting the two curves in Fig. 1 represents $a_s$, i.e. the value of $a$ used in comparing the unknown and standard solutions. $a_s$ equal to 9 has been used throughout the present work. In Fig. 1 we find by interpolation that 0·16 ml. blood extract is equivalent to 0·245 ml. standard $V$-factor solution (for preparation and concentration, see below), each producing a change equal to $a_s$. From these data $X$ and $S$ are calculated, and knowing $H$ the value of $V$ is obtained from (2).

*Blood extract.* Blood obtained by venepuncture is prepared by the addition of 1·2 mg. potassium oxalate per ml. 0·1 ml. blood is added to 10 ml. distilled water. Immediately 2 ml. are withdrawn for the haemoglobin determination [Evelyn, 1936] and are replaced by 2 ml. trichloroacetic acid solution (13% acid containing 2·15% NaOH). The test tube is plugged, centrifuged after several minutes and stored on ice. The $V$-factor is contained in the supernatant liquid, which loses 5% of its activity in 24 hr. Plasma prepared in this manner supports no measurable growth, even when 1 ml. instead of 0·1 ml. is used.

### *Cultivation of bacteria*

The bacteria used were of the same strain as those employed by the Lwoffs, namely, *H. parainfluenzae* strain 4101 Fleming, National Collection of Type Cultures, Lister Institute, London. Subcultures were made at least three times a week, alternating between broth and agar. When first received, the bacteria showed 50–100% more growth if glucose or sucrose were added to the medium. After 3½ months of cultivation at 37°, however, this phenomenon disappeared rather suddenly, although the ability to oxidize sugar was retained. All data reported here were obtained after the mutation had occurred; during this period cultivation in broth was almost continuous.

*V-factor.* This is prepared by suspending 100 g. baker's yeast in 50 ml. water, pouring the suspension into 150 ml. boiling water containing 6·8 g. $KH_2PO_4$, and maintaining the temperature at 80–85° for 20 min. The suspension is then filtered through a bacteriological paper, and sterilized by passing through a candle or a Seitz filter pad. The concentration of this solution is $5 \times 10^{-1}$, as 0·5 g. yeast per ml. water has been used. The strength of the *standard solution* referred to in the text and used in all experiments was $6 \times 10^{-4}$.

*Medium.* A 2% solution of proteose-peptone (Difco) containing 0·6% NaCl is titrated with NaOH to *p*H 7·7–7·8, tubed in 6 ml. samples, and autoclaved. Sterile *V*-factor is then added. The usual subculture receives 0·1 ml., concentration $5 \times 10^{-2}$. Subcultures intended to supply inocula for blood tests receive 0·1 ml., $2{\cdot}5 \times 10^{-3}$, all of which will be used up after 24 hr. of growth. Thus when the test culture is inoculated with one drop, no *V*-factor will be carried over. For a solid medium, the above is used plus 3% agar (*V* added when agar has cooled, but before solidification).

## Experimental results

Figs. 2, 3 and 4 together with Table I summarize the experimental data. The solid black points are plotted for periods during which nicotinic acid was taken; an arrow indicates when the administration of acid began or ended between two points; *V* the ordinate in each graph was calculated according to equation (2), *H* and *S* being redetermined for every point. The abscissae of all are comparable; i.e. a determination made on the twentieth day of Fig. 2 would have been made simultaneously with that on the twentieth day of Fig. 3. This was done to provide a further check on the method.

All normal subjects took nicotinic acid by mouth after breakfast and dinner either in tablets (Squibbs) or in solution (S.M.A. Corp. nicotinic acid dissolved in water). A 0·5% solution (partially neutralized) has a slightly sour taste and is not unpleasant. When 100 mg. are taken on an empty stomach (e.g. before breakfast) a peripheral flushing occurs, the extent of which varies in different individuals. Usually within 5 min. the face and neck tingle, then flush, followed by the forearms and probably the knuckles and knees. In some cases intravenous injection was used.

### *Normal subjects*

(1) HK (Fig. 2), white male, age 28, height 179 cm., weight 66·5 kg. During first 10 days, average *V* was 19. From 10th to 24th day about 100 mg. nicotinic acid were taken daily, the change in *V* being as follows: 16th day, total nicotinic acid 475 mg., +20%; 19th day, 825 mg., +40%; 22nd day, 1500 mg., using smooth curve +50%.

Beginning with the 24th day nicotinic acid was discontinued, and the subject was placed on a pellagra-producing diet (defined below). The increase in *V* declined: 32nd day, +10%; 40th day, +0%. Beginning on the 43rd day 1200 mg. nicotinic acid were taken within 24 hr. (300 mg. at 11.30 a.m., 2 p.m., 6.15 p.m. and 10.30 a.m.): 8 hr. after first dose *V* rose +10%; 24 hr. (44th day), +40%; 48 hr. (45th day), +50%; 5 days (48th day), +30%; 9 days (52nd day), +5%.

The pellagra diet used was similar to that of Ruffin & Smith [1937], but with the following changes: *omitted*, cod liver oil, iron ammonium citrate, calcium gluconate, field (cow) peas, lard and flour; *decreased*, ascorbic acid to 50 mg. per day; *added* per day, 1 mg. aneurin-Cl; 5 drops oleum percomorphum, 200 g. potatoes, 5 stewed prunes, 70 g. apple sauce, 20 g. butter. The modified diet, as compared with the original, contains less preventive substance [cf. food ratings of Sebrell, 1934], but is low in protein.

(2) RM (Fig. 3, open circles), white male, age 24, weight 70 kg. Average initial $V$ was 17 during the 13th–16th days. During 16th–23rd days about 100 mg. nicotinic acid taken daily. The changes in $V$ together with the total dose were: 20th day, 400 mg., +30%; 23rd day, 1 g., smooth curve +35%; thereafter nicotinic acid stopped, 34th day, −5%. Beginning on the 50th day 1·25 g. nicotinic acid were taken during 24 hr. The changes in $V$ were: 50th day (after 250 mg.), +10%; 52nd day, +30%; 55th day, +25%.

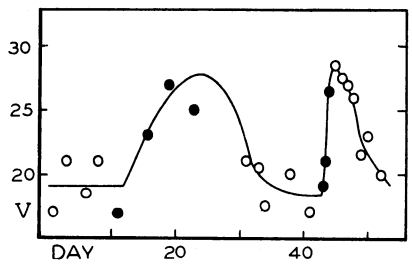


Fig. 2.

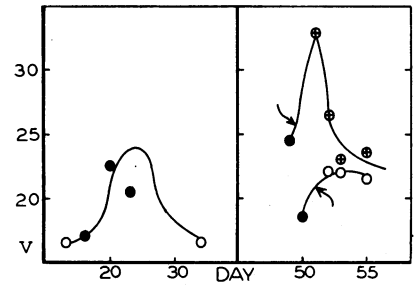


Fig. 3.

Fig. 2. Variation of $V$ in the normal subject HK. 1·5 g. nicotinic acid taken during the 10th–24th days produced an increase of 50%. 1·2 g. taken within 24 hr. on the 43rd day produced a similar effect. Solid circles indicate periods during which nicotinic acid was taken.

Fig. 3. Variation of $V$ in the normal subject RM (open circles) and the pellagrin JH (crossed circles). Solid circles indicate the administration of nicotinic acid, which was stopped at the arrows. RM (left curve) received 1 g. nicotinic acid in 7 days during which $V$ rose 35%. Later (right-hand curve) RM received 1·25 g. in 24 hr., and $V$ rose 30%. JH the pellagrin received 700 mg. nicotinic acid within 48 hr. ending at the arrow. Note the rapid rise in $V$ of 35%, and the rapid fall.

The remaining four white male subjects, whose data are graphed together in Fig. 4C, will be considered as a group. Ages, weights and graph symbols are as follows: FE, 23, 66, circle; GS, 24, 61·5, triangle; KB, 22, 79·5, square; WK, 22, 97·5, inverted triangle. The daily dose of acid was about 1·5 mg. per kg.; FE and GS took 100 mg. per day, KB 125 and WK 150.

The data show an average initial $V$ of 18 during the 13th–16th days. About 1·5 mg. nicotinic acid per kg. were taken daily during the 16th–20th days, and twice this dose for the 21st and 22nd days. The increases in $V$ were: 20th day, 40%; 23rd day, 45%; 35th day, 10%.

## *Pellagrins*

(1) JH, white male, age 55, weight 40 kg. The patient had suffered from oesophageal obstruction for 25 years. For 6 months before admission almost all food eaten had been regurgitated within 5–20 min.; as a result he was quite emaciated. About 2 weeks before admission definite signs of pellagra had appeared. On admission, physical examination showed the dorsum of each hand to be red and rough, and the tibial surfaces of the legs to be discoloured with a brownish pigmentation. The lips were dry, the tongue very red and smooth, the buccal surfaces red, the pharynx red. Oesophageal dilatation was performed with marked relief of the obstruction, and the patient was put on a high caloric, high vitamin diet. Valentine's liver extract, 30 ml., t.i.d., and nicotinic acid, 20 mg., t.i.d., were given for 4 days, then stopped. On the 5th and 6th days 300 and 400 mg. nicotinic acid were given. Eight days later acid and liver extracts were restored to the diet.

Data for JH are plotted in Fig. 3, crossed circles; the arrow marks the end of 2 days during which 700 mg. nicotinic acid were taken. At the time of the first test 600 mg. had been taken already, and $V$ was 24·5 (49th day on graph). Note the subsequent sharp rise and fall: +35%, 51st day; +8%, 52nd day. The true initial value of $V$ is not known; hence the increase (see Table I) is a minimum. The patient's course was one of steady improvement. The signs of dermatitis disappeared almost entirely within 3 weeks.

The following cases were kindly brought to my attention by Drs D. T. Smith and J. M. Ruffin; they constitute part of a series studied in other respects by these workers, which will be described

elsewhere. The basal diet referred to [Ruffin & Smith, 1937] contained no vitamin fortification and a minimum of *pp* factor.

(2) RC, white male, age 41, weight 61·9 kg., height 176 cm. Three weeks before admission, after exposure to sun, dermatitis developed on his hands, and was followed 1 week later by stomatitis and glossitis. Diet was fair, but lacking in meat. There was no history of alcoholism. On admission, physical examination showed the skin of the dorsa of the hands to be reddened, cracked and peeling. "Sand paper skin" was noted on both sides of the nose around the sebaceous glands. The gums were sore and tender, the mucous membranes, pharynx and tongue were red. Gastric analysis revealed no free HCl at any time. Gastroscopy showed a reddened mucosa without ulcers or erosions.

RC was placed on the basal diet and given graduated doses of sunlight to the right hand. After 3 days his tongue became red and swollen, the dorsum of the hands inflamed, and a mild diarrhoea developed. During this period the average *V* (Fig. 4B, circles) was 15. Nicotinic acid administration then began (3rd day in Fig. 4B), 90 mg. per day i.v. After the second dose an improvement could be noted in the glossitis, and from then on the patient improved steadily, although the sebaceous glands over the nose were tardy in responding. According to Fig. 4B the increases in *V* correlated to total dose of nicotinic acid were: 6th day, 270 mg., 40%; 9th day, 540 mg., 75%; 12th day, 810 mg., 85%.

In Fig. 4A there are compared the results obtained with two subjects on the same hospital diet and with same initial value of *V*, one of whom received nicotinic acid.

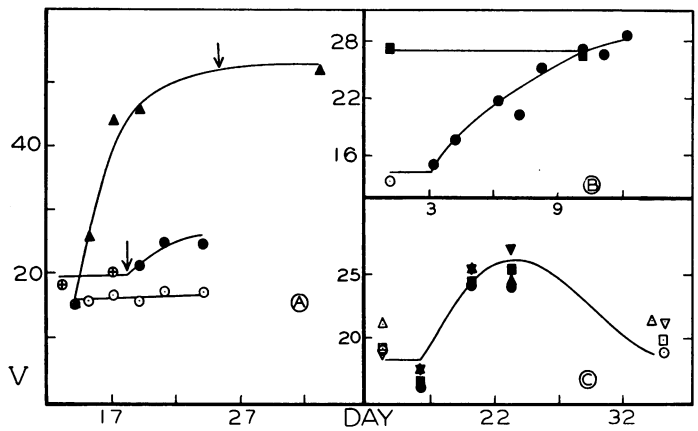


Fig. 4. Variation in *V* following the administration of nicotinic acid (indicated by solid circles and arrows). Fig. 4A, triangles, pellagrin JS, 1 g. daily, *V* rose 250%; crossed circles, pellagrin LL, 60 mg. daily, *V* rose 30%; open circles, pellagrin RJ, spontaneous recovery, *V* rose 5%. Fig. 4B, circles, pellagrin RC, 90 mg. daily, *V* rose 100%; squares, pellagrin JA, received 5·6 g. during 26 days before first determination, and a total of 8·8 g. at time of the second. Fig. 4C, normal subjects, circle, FE; triangle, GS; square, KB; inverted triangle, WK. Each took 1–1·5 g. nicotinic acid. The average rise in *V* was 45%.

(3) RJ, white male, age 55, weight 61·3 kg., height 169 cm. The diagnosis was pellagra, hypertensive cardiovascular disease and arteriosclerosis. Inquiry revealed that he had had an apoplectic seizure 17 months previously, following which his physician had ordered the elimination of meat from the diet. There was also a lack of fruit. Two weeks previously after exposure to the sun for several hours the signs of pellagra had appeared. When admitted, the dorsal skin of each hand was fiery red and desquamating, with oedema of the subcutaneous tissue. The bowels were costive. Glossitis was not present.

The data for RJ are plotted in Fig. 4A, open circles. When admitted, he was placed on the basal diet and exposed to the sun for several days without effect. The dermatitis improved steadily, and he was finally discharged some 20 days later. The initial value of *V* was 15, and it remained at this level, advancing to 16, an increase of about 5%. The spontaneous recovery may have resulted in part from the eating of a meal of liver following the outbreak of the dermatitis.

(4) JS, white male, age 55, weight 59·9 kg., height 158 cm. The diagnosis was pellagra, hypertensive vascular disease, generalized arteriosclerosis and peripheral neuritis. About 2 years previous to admission, the patient had been treated at this hospital for pellagra with Valentine's liver extract, with excellent results. Upon returning home, however, medication was stopped and meat was eliminated from the diet because of the high blood pressure. One month before admission, he had strolled through the fields in the sun; shortly afterwards dermatitis, stomatitis and diarrhoea developed. Upon admission, dermatitis of the dorsum of the hands was noted, the lips were swollen and sore, the mucous membranes red and dry, the tongue bright red and very slick and the pharynx reddened. There was no diarrhoea, but the patient complained of gastrointestinal symptoms, which continued throughout his course in the hospital. Gastric analysis showed the presence of free HCl after histamine. The patient was put on the basal diet, and 2 days later the oral administration of nicotinic acid was begun, 250 mg., q.i.d. Beginning 3 days after admission, and continuing for 3 successive days, 100 mg. per day of aneurin-Cl were given.

Data for JS are plotted in Fig. 4A, triangles. The initial value of $V$ was 15; graph abscissa, 14th day. Thereafter the total dose of nicotinic acid and increases in $V$ were: 15th day, 1 g., 75%; 18th day, 4 g., 190%. Administration of nicotinic acid was stopped on the 25th day, total dose, 11 g. On the 33rd day, the increase in $V$ was 245%; probably the increase was even greater around the 27th day.

(5) JA, white male, age 65, weight 56·6 kg., height 177·5 cm. Diagnosis: pellagra, generalized arteriosclerosis, polyneuritis due to beriberi. At time of admission this patient was critically ill. He was afflicted with a marked psychosis, the lesions on his hands were secondarily infected and the stomatis and glossitis were marked. He was put on the basal diet and given nicotinic acid, 90 mg. i.v. daily for 10 days. At the end of this period there was an amazing improvement in his entire condition. Nicotinic acid was then given orally, 90 mg., q.i.d., and also aneurin-Cl.

The first value of $V$ for JA (Fig. 4B, squares) was obtained 26 days after treatment had been begun, at which time the patient had received 5·6 g. nicotinic acid and 450 mg. aneurin-Cl; $V$ was

Table I. *Effect of nicotinic acid administration on* V

| Subject | Total acid taken | Period of administration | V | | | Duration of increase |
|---|---|---|---|---|---|---|
| | | | Initial | Maximum | Increase | |
| Normals | mg./kg. | days | units | units | % | days |
| HK* | 22·5 | 10 | 19 | 28 | 50 | 10 |
| | 18 | 1 | 19 | 28·5‡ | 50 | 8 |
| JM | 14·5 | 7 | 17 | 23·5 | 35 | 11 |
| | 18 | 1 | (17) | 22‡ | 30 | — |
| Average of FE, KB, GS WK | 14·5 | 7 | 18 | 26·5 | 45 | 8 |
| Pellagrins | | | | | | |
| JH | 17·5 | 2 | Less than 24·5 | 33§ | More than 35 | 2 |
| RC† | 4·3 | 3 | 14 | 21 | 50 | — |
| | 8·6 | 6 | — | 26 | 85 | — |
| | 13 | 9 | — | 28 | 100 | — |
| JS† | 17 | 1 | 15 | 26 | 75 | — |
| | 67·5 | 4 | — | 44 | 190 | — |
| | 186 | 11 | — | 52‖ | 254 | — |
| LL | 8 | 6 | 19 | 25 | 30 | — |
| JA | 215 | 26 | — | 26·5 | — | — |
| | 250 | 35 | — | 26·5 | — | — |

* After taking first dose of nicotinic acid the subject was maintained on a pellagra-producing diet.

† On Ruffin & Smith basal diet.

‡ 24 hr. after nicotinic acid was discontinued.

§ 36 hr. after nicotinic acid was discontinued.

‖ 8 days after nicotinic acid was discontinued

about 26·5; 9 days later the value was the same, the total doses being 8·8 g. nicotinic acid and 850 mg. aneurin-Cl.

(6) LL, white female, age 40, weight 45·8 kg., height 160 cm. Diagnosis: malnutrition, pellagra, secondary anaemia, pyorrhea alveolaris, chronic cervicitis. Three months before admission there had been a gradual onset of weakness, anorexia and proneness to fatigue; 3 weeks before admission dermatitis began. When admitted, the skin of the face was somewhat reddened, and on the hands and arms to just over the elbows it was rough, brownish and scaling. Similar lesions were present on the ankles.

LL was put on the basal diet and exposed to sunlight, but showed no sensitivity. The data for $V$ are plotted in Fig. 4A, crossed circles. The initial value was about 19; after 6 days on a high caloric, high vitamin diet plus 60 mg. nicotinic acid per day (begun at arrow), $V$ had risen 30%. The patient's general condition improved and she was discharged.

## Discussion

The results presented, which are plotted in the figures and summarized in Table I, establish that $V$ is a direct function of nicotinic acid intake. The normal value is about $18 \pm 2$, and this was increased by as much as 200% following the ingestion of nicotinic acid. Within the range of dose studied (about 20 mg. per kg.), the increase in $V$ is independent of the duration of the period of administration (1–10 days).

It is rather surprising, however, that $V$ should fall so rapidly to the original basal level when nicotinic acid is discontinued, particularly since no measurable quantities are detected in the plasma. Apparently some fundamental difference exists between the initial $V$-factor found in the corpuscle and the increment which is rapidly added. The basal level is stable and possibly is determined entirely at the time of haematopoiesis, whereas the increment is mobile and reflects the current state of nutrition.

Whether these levels can be used to indicate pellagrous conditions is not certain. It is suggestive that the duration of the increment in the pellagrin JH was 2 days, whereas in the normals it was about 10. The relation between the basal level and pellagra is certainly complicated. For example, RJ and JS (Fig. 4A) had the same initial $V$ although the symptoms of JS were very much the worse; RJ made a spontaneous recovery. The few cases reported taken with several others indicate that pellagra is to be suspected when $V$ is not more than 15, although the converse may not be true.

No relation between $V$ and haemoglobin was noted.

If we accept nicotinic acid as the vitamin whose deficiency causes pellagra, a number of consequences follow.

(1) In pellagra the coenzyme level of the body will be reduced, leading directly to a reduced capacity for oxidations and reductions. The basal metabolism will not necessarily be affected, however, since a fraction of the total coenzyme should be adequate for it, but the maximum rate of work will be decreased.

(2) The more active the organism, the greater will be its nicotinic acid requirement. This conclusion is attractive because it will explain some of the spontaneous recoveries, and also because it partly accounts for the differential susceptibility of individuals on the same diet.

The coenzyme role of nicotinic acid, however, does not appear capable of explaining all of the facts relating to pellagra. As noted previously, the relationship between blood $V$-level and pellagra is not clear. Furthermore, since these experiments were completed, Daft *et al.* [1938] in a preliminary note have

reported that 50 mg. of Co I administered intravenously failed to have a therapeutic effect on black tongue. Thus it seems probable that nicotinic acid enters the metabolism in ways as yet unknown.


## Summary

The bio-assay of blood *V*-factor was accomplished by means of *Haemophilus parainfluenzae*, as supplied by Lwoff & Lwoff. *V*-factor represents the coenzyme moiety (di- and tri-phosphopyridine nucleotides), and, possibly, closely related substances which are unknown. Nicotinic acid (or amide) does not give the test.

*V*-factor is confined to the corpuscles. About 20 mg. nicotinic acid per kg. taken in 1–10 days will increase *V* by 35–75% in 2–10 days in normal individuals and pellagrins.

The rapid rise in *V*-factor is followed by a fairly rapid fall to the original level. Apparently, material responsible for the increment in *V* is to be distinguished from that which constitutes the initial corpuscular content.


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