

# On the Vitamin B Complex Content of the Hair of Normal and Pellagrous Subjects

HUGUES GOUNELLE, M.D.,\* MILAN MITROVIC, M.D.† AND MICHEL DEMARNE, M.D.

THE protective role played by most of the B-complex vitamins on the epidermis is known. We thought it would be of interest to investigate the content of such vitamins in the hair of pellagrous subjects. However, we had no data on the hair content of normal subjects. Therefore, we had to determine the values in control subjects immediately after cutting their hair and after several months of conservation. This second determination was indispensable for interpreting the values found in pellagrous subjects, which were determined several months after taking the sample.

The B-complex vitamins quantitatively determined were niacin, riboflavin, pyridoxine, and pantothenic acid. The determinations were made at the Foch Research Center in Paris, under the supervision of Mlle Marnay.

## NIACIN

We used the method of Snell and Wright,<sup>1</sup> i.e., opacimetric measurement of the growth of *Lactobacillus arabinosus*. The fat is removed from finely cut hair by means of two or three applications of chloroform, with agitation for fifteen minutes each time. The sample is left in the dryer overnight, under vacuum. To a sample of 100 mg. of hair from which the fat has been removed are added 15 ml. N HCl; this solution is passed through the autoclave at 120°C. for sixty minutes and, after filtration, the pH is brought to 6.8 and the suitable dilutions prepared.

From the Centre de Recherches Foch, Centre de Recherches Cliniques et Biologiques sur la Nutrition de l'Homme, Paris, France.

\* Director; † Present address: U.I.N. arodni Front 46-48, Belgrade, Yugoslavia.

This study was supported by a grant from the Institut National d'Hygiene.

*Content of Hair from Normal Subjects.* We made twenty-five determinations on apparently healthy subjects between two and ninety-five years of age. No treatment likely to have included nicotinamide had been prescribed for them recently. Table I shows the results obtained, which ranged from 7 to 43  $\mu\text{g.}$  of nicotinic acid/gram of hair, the average being 18  $\mu\text{g.}$  Color, thickness and age did not appear to have any significant bearing on these data.

*Content after Three Months of Storage.* Quantitative determinations were carried out on nine samples a short time after cutting; these were then kept in locks at room temperature, in the dark, and tested a second time three months later.

The following results were obtained: before storage, 20, 18, 13, 7, 9, 18, 24, 7 and 12  $\mu\text{g./gm.}$  of hair; after storage, 19, 16, 13, 8, 9, 18, 25, 7 and 14  $\mu\text{g./gm.}$  of hair.

Storage did not result in any reduction in nicotinic acid content. The figures remain constant, and the initial average of 14  $\mu\text{g./gm.}$  is found to be almost exactly the same after three months.

*Content of Hair from Pellagrous Subjects.* The samples were taken from subjects with pellagra, which was rampant in Yugoslavia and affected Albanian and Serbian subjects. The quantitative determination was performed three months after cutting. Table II gives the results obtained in the twenty-five determinations. The arithmetic average is 9  $\mu\text{g./gm.}$  of hair.

This figure is therefore significantly lower as compared to that obtained under similar conditions for the hair of healthy French subjects. The hair of pellagrous subjects, therefore, actually seems to have a lower nicotinic acid

TABLE I  
Niacin Content of the Hair of Normal Subjects

Age (yr.)	Sex	Color	Texture	Niacin Content ( $\mu\text{g./gm. of hair}$ ) (fat removed)
2	M	Blond	Very fine	31.5
6	F	Dark brown	Fine	9.7
11	M	Dark brown	Fine	13
16	F	Dark brown	Thick	13.5
24	M	Dark brown	Fine	14.1
26	F	Very dark brown	Rather fine	20.7
26	M	Black	Medium	24.3
26	F	Light brown	Medium	18.6
29	M	Dark brown	Thick	20.4
38	M	Brown	Rather fine	27
46	M	Brown (some white)	Rather fine	20
47	M	Black	Rather thick	24
49	F	Dark brown	Very fine	9.4
60	F	Dark brown (some white)	Rather fine	18.6
64	F	Very dark brown	Fine	18.7
74	F	Gray	Rather fine	9.2
74	F	Black (some white)	Medium	43
74	F	Brown	Very fine	7.3
75	F	Black (some white)	Medium	37.1
76	F	Light brownish blond (some white)	Rather fine	7.5
83	F	Dark brown (some white)	Fine	10.1
83	F	White	Fine	7.4
85	F	Dark brown and some white	Rather fine	17.7
85	F	Dark brown and some white	Very fine	16.8
92	F	White and light brown	Rather thick	12.4
Average				18

content. If the results are analyzed in detail, it is observed that there are normal or even high concentrations, but that the average remains appreciably lower than in normal subjects.

#### RIBOFLAVIN

We used the microbiologic method of *Lactobacillus casei* growth and quantitative determination of the lactic acid formed according to the technic of Snell and Strong.<sup>2</sup> The hair underwent the same preparation as for the preceding determinations. After drying under vacuum, a sample was placed in 0.1 N HCl and passed through the autoclave for twenty minutes at 120°C.

*Content of Hair from Normal Subjects.* In fifteen subjects the values are quite constant and range from 1  $\mu\text{g./gm.}$  of hair, the lowest concentration, to 2.6  $\mu\text{g./gm.}$  of hair, the highest concentration. The most prevalent concentrations lie between 1.2 and 2

$\mu\text{g./gm.}$  Neither the effect of thickness or pigmentation of the hair, nor that of the age of the subject, was found to be of any significance.

*Content after five months of storage.* The quantitative determination of riboflavin in seven samples kept in the dark for five months yielded the following results: before storage, 1.2, 2.5, 2.6, 2.2, 1.6, 2, and 2  $\mu\text{g./gm.}$  of hair; after storage, 0.5, 1.2, 1.2, 0.9, 1.2, 0.2 and 0.8  $\mu\text{g./gm.}$  of hair; the average being 2  $\mu\text{g./gm.}$  of hair before storage and 0.8  $\mu\text{g./gm.}$  of hair after storage.

Storage was therefore found to result in a general decrease in the second determination; this decrease is, on the average, 60 per cent of the initial concentration.

*Content of Hair from Pellagrous Subjects.* The hair was collected in July and August 1958 but could not be quantitatively determined in Paris until five months later. The nineteen determinations made are recorded in

TABLE II  
Niacin Content of Hair from Pellagrous Subjects (After Three Months of Storage)

Age (yr.)	Sex	Color	Texture	Nicotinic Acid Content ( $\mu\text{g. gm. of hair}$ ) (fat removed)
1	M	Golden blond	...	6.2
5	M	Light blond	Fine	8.4
8	M	Light brown	Medium	4.1
8	M	Golden blond	...	7.4
12	F	Auburn	...	7.3
12	M	Brown	Medium	6.2
16	M	Brown	Medium	8.9
17	M	Dark brown	...	14.5
20	F	Titian red	...	4.9
20	F	Auburn	Medium	5.4
24	F	Brown	Fine	8.9
30	F	Black	Thick	21.6
40	F	Brown	Fine	4.5
40	F	Auburn	Medium	8.8
40	F	Dark brown (some white)	Fine	12.1
45	F	Dark brown	...	6.2
45	F	Black	Medium	17.3
48	F	Dark brown (some white)	...	15.5
50	F	Black	Thick	13.2
50	F	Dark brown	Medium	19.3
60	F	White (some dark brown)	...	5.4
60	F	Auburn	Thick	5.4
60	F	Auburn and white	Fine	11.6
65	F	Brown	Medium	10.1
70	F	Golden brown	Medium	11.9
Average				9

Table III. It will be noted that certain figures are especially high, accounting for a higher general average than that determined in the control subjects after storage for the same length of time. Indeed, the average obtained in the pellagrous subjects was 1.5  $\mu\text{g./gm.}$  If it is considered that this hair lost 60 per cent of its initial content during the five months following its cutting, we are led to conclude that this hair had a higher riboflavin content than that of the control subjects.

Before concluding that this increase is related to the pellagrous condition, however, it would be necessary to eliminate several other causes and clarify certain points. Does the diet of the Yugoslav and the French subject provide the same vitamin content? Moreover, investigation showed that B-complex vitamins had been prescribed for many of these pellagrous subjects. In any event, we found no

evidence of riboflavin deficiency in pellagrous hair.

#### PYRIDOXINE

We used the microbiologic method of determination by means of opacimetric measurement of the growth of *Saccharomyces carlsbergensis*, according to the technic of Atkin, Schultz, Williams and Frey.<sup>3</sup>

*Content of Hair from Normal Subjects.* Twenty-seven determinations were carried out on subjects in all age groups, from eight months to eighty-seven years. The results, shown in Table IV cover a rather wide range, with the concentrations varying from 0.07 to 0.46  $\mu\text{g./gm.}$  of hair; the intermediate concentration was, on the average, about 0.20  $\mu\text{g.}$  It is difficult to establish a relationship with the age of the subject, or the color or thickness of his hair.

TABLE III  
Riboflavin Content of Hair from Pellagrous Subjects After Five Months

Age (yr.)	Sex	Onset of Disease (yr.)	Color	Texture	Vitamin Content ( $\mu\text{g./gm. of hair}$ ) (fat removed)
6	F	...	Golden blond	Fine	0.93
7	F	...	Blond	Fine	0.40
9	M	...	Medium brown	Medium	0.42
13	M	...	Medium brown	Fine	0.56
18	M	...	Dark brown	Thick	0.64
20	M	3	Medium brown	Medium	1.90
24	M	3	...	...	2.56
30	F	5	Dark brown	Fine	1.35
30	F	1	Black	Thick	2.15
30	F	5	...	...	1.79
33	F	7	Jet black	Thick	3.32
39	F	10	Dark brown	Thick	3.80
40	F	3	Black	Thick	1.41
43	F	10	Dark brown	Fine	2.89
45	F	5	Dark brown	Medium	2.81
50	F	3	Dark brown	Very fine	1.03
60	F	10	Dark brown	Rather fine	1.12
65	F	3 wk.	Medium brown	Thick	0.17
67	F	5	White	Thick	0.46
Average					1.5

*Content after seven months of storage.* After seven months of storage at room temperature, in the dark, some samples were again subjected to quantitative determinations, which yielded the following results: before storage, 0.46, 0.12, 0.19, 0.13 and 0.11  $\mu\text{g./gm. of hair}$ ; after storage, 0.08, 0.03, 0.06, 0.02, 0.02 and 0.02  $\mu\text{g./gm. of hair}$ .

The average figure after cutting was 0.18  $\mu\text{g./gm. of hair}$ , which dropped to less than 0.04 after seven months. The loss is therefore considerable, i.e., about 80 per cent.

*Content of Hair from Pellagrous Subjects.* The determinations were carried out on thirteen samples seven months after cutting. The results, given in Table v, range from 0.06 to 0.46  $\mu\text{g./gm. of hair}$ , the average being 0.15  $\mu\text{g.}$

The average obtained is almost the same as that observed in hair from normal subjects. Actually, if an 80 per cent loss is assumed after seven months, as indicated, the initial content of hair from pellagrous subjects would be higher than that determined in normal subjects. However, the same reservations

must be made as in the case of riboflavin. We have no comparative data regarding French and Yugoslav control subjects, and the administration of vitamins could have been a factor in some cases. Nevertheless, the hair of pellagrous subjects does not seem to be deficient in pyridoxine.

#### PANTOTHENIC ACID

Pantothenic acid is of particular interest since, in certain pathologic conditions, it exerts a favorable effect on hair growth. We used the microbiologic method of L. arabinosus growth developed by Skeggs and Wright,<sup>4</sup> determining the free pantothenic acid content. Petroleum ether was used for the fat removal; extraction was effected after grinding with Fontainebleau sand (about 1,500 mg./100 mg. of hair from which the fat was removed) and washing in the autoclave with distilled water for fifteen minutes at 120°C. After filtration, the pH was adjusted to 6.8 and the suitable dilutions were prepared.

*Content of Hair from Normal Subjects.* A study by Stangl<sup>5</sup> in 1952 established the

TABLE IV  
Pyridoxine Content of Hair of Control Subjects

Age (yr.)	Sex	Color	Texture	Pyridoxine Content ( $\mu\text{g./gm. of hair}$ ) (fat removed)
8/12	M	Brown	Fine	0.24
9/12 <sub>4</sub>	M	Ash blond	Very fine	0.29
1 <sup>1</sup> / <sub>2</sub>	F	Light blond	Very fine	0.07
3	M	Light brown	Very fine	0.46
5	M	Auburn	Fine	0.35
15	F	Auburn	Fine	0.32
22	F	Dark brown	Thick	0.42
24	M	Dark brown	Fine	0.22
26	M	Dark brown	Thick	0.41
30	F	Jet black	Medium	0.13
30	F	Dark brown	...	0.33
32	F	Black	Medium	0.12
35	F	Black	...	0.29
51	F	Black	Medium	0.32
55	F	Black	Very fine	0.14
57	F	Dark brown	Fine	0.25
68	M	White	Fine	0.19
71	F	Gray	Fine	0.19
75	F	Silver-white	Fine	0.22
77	F	White	Very fine	0.13
79	F	Gray	Fine	0.42
79	F	Blond	Very fine	0.12
80	F	White	Fine	0.11
83	F	Gray	Medium	0.36
84	F	Black	...	0.09
85	F	White	...	0.17
87	F	Gray	Thick	0.21
Average				0.22

presence of free pantothenic acid in concentrations of 0.05 to 2  $\mu\text{g./gm. of hair}$ . Our own study covers thirty-eight control subjects, the results obtained being listed in Table VI.

The range of concentrations we found is somewhat less broad than that reported by Stangl. They extend from 0.43 to 2.99  $\mu\text{g. of free pantothenic acid/gram of hair}$ , the majority of the concentrations being between 0.60 and 2, with an average of 1.3  $\mu\text{g./gm.}$

The subjects were between sixteen months and eighty-nine years of age, and their hair color varied. We did not find age or color or thickness of the hair to be of any particular significance. In passing, we might note the relatively high content of free pantothenic acid in hair as compared with that found in the blood. In fact, Gounelle and Richet<sup>6</sup> found an average concentration of 4.6  $\mu\text{g./100 cc. of serum}$ , which corresponds to a content approximately twenty-five times lower than that of the hair.

*Content after three months of storage.* On the whole, three months of storage in the dark,

TABLE V  
Pyridoxine Content of Hair from Pellagrous Subjects (Seven Months After Cutting)

Age (yr.)	Sex	Color	Texture	Pyridoxine Content ( $\mu\text{g./gm. of hair}$ ) (fat removed)
12	F	Golden blond	Fine	0.06
24	F	Jet black	Thick	0.08
28	F	Black	Medium	0.08
30	F	Brown	Very fine	0.19
30	F	Dark brown	Fine	0.20
31	F	Dark brown	Fine	0.21
34	F	Dark brown	Fine	0.20
50	F	Auburn	Medium	0.08
50	F	Black and white	Medium	0.07
60	F	Dark brown	Fine	0.18
60	F	Light brown	Medium	0.46
60	F	Auburn	Medium	0.07
65	F	Black	Thick	0.09
Average				0.15

at room temperature, is followed by a loss in content. We tested nine samples, the average figure obtained being 1.1  $\mu\text{g./gm. of hair}$  at the beginning and 0.82  $\mu\text{g./gm.}$  after three months, i.e., a decrease of about one fourth. However, this drop is not constant; thus, the first two samples maintained practically unchanged concentrations, and for sample No. 8, the loss was only 10 per cent.

The following results were obtained: before storage, 1.07, 1.13, 2.42, 1.56, 0.90, 1.09, 0.83, 0.73 and 0.72  $\mu\text{g./gm. of hair}$ ; after storage, 1.01, 1.24, 1.78, 0.79, 0.50, 0.71, 0.55, 0.67 and 0.49  $\mu\text{g./gm. of hair}$ .

*Content of Hair from Pellagrous Subjects.* The hair of pellagrous Yugoslav subjects was obtained in September 1958, but the determinations were not made until three months later.

The concentrations obtained, shown in Table VII, were between 0.5 and 2.88  $\mu\text{g. of free pantothenic acid/gram of hair}$ , with an average of 1.3  $\mu\text{g.}$  Here, too, we did not find the age of the subject or the color or thickness of his hair to be of any particular significance.

The value of 1.3  $\mu\text{g.}$  shows that there is no decrease in the free pantothenic acid content of pellagrous hair. Taking into account the storage period which, as we have seen, generally results in a reduction in concentration, it is conceivable that the initial concentration

TABLE VI  
Free Pantothenic Acid in Hair of Normal Subjects

Age (yr.)	Sex	Color	Texture	Content ( $\mu\text{g./gm.}$ of hair)
11 $\frac{1}{2}$	F	Blond	....	0.98
11 $\frac{1}{2}$	F	Light brown	....	0.97
11	F	Dark brown	Fine	1.54
12	F	Blond	Fine	0.74
15	F	Blond	Fine	1.18
19	M	Light brown	Fine	2.01
19	F	Black	....	1.94
19	M	Dark brown	Thick	2.21
19	M	Dark brown	Thick	2.23
19	F	Dark brown	Thick	2.45
19	F	Jet black	Thick	2.99
22	F	Dark brown	Thick	2.13
22	M	Ash brown	Medium	1.74
22	F	Blond	Fine	0.85
24	F	Black	Thick	1.13
25	F	Dark brown	Medium	1.04
25	F	Dark brown	Thick	0.63
25	F	Black	....	0.74
26	F	Ash blond	Fine	1
26	M	Dark brown	Thick	2
26	F	Medium brown	Fine	0.51
28	M	Black	Medium	0.81
28	F	Dark brown	....	1.12
29	M	White and much black	Thick	1.07
29	F	Jet black	Thick	0.83
30	F	Medium brown	Fine	0.84
30	F	Black	Medium	1.34
31	F	Ash blond	Fine	0.43
70	F	Gray	Thick	0.73
76	F	White, some black	Thick	1.09
82	M	White, some black	Fine	1.56
82	F	White, some black	Medium	0.99
83	F	White, some black	Thick	0.81
85	F	Very white	Fine	1.14
86	F	Dark brown	Fine	0.83
88	F	White, some black	Medium	2.42
89	F	Ash gray	Medium	0.72
Average				1.28

might have been higher, although still within normal limits.

#### CONCLUSIONS

We determined the content of four B-complex vitamins in hair from pellagrous subjects, first determining the content of these same vitamins in the hair of normal subjects. Since the determinations in the case of the pellagrous subjects could not be made until several months after the samples were taken, a comparative determination was carried out after a similar period of storage of the control hair.

The niacin content of hair from pellagrous subjects was found to be definitely lower in comparison with that of the control subjects.

TABLE VII  
Free Pantothenic Acid in Hair of Pellagrous Subjects

Age (yr.)	Color	Texture	Content ( $\mu\text{g./gm.}$ of hair)
40	Black	Fine	1.14
27	Dark brown	Fine	1.36
25	Medium brown with glints of red	Fine	2.17
37	Brown	Fine	0.91
80	Very light blond	Very fine	2.88
80	Dark brown and white	Very fine	0.81
30	Dark brown	Fine	2.71
5	Golden blond	Fine	0.61
65	Black and white	Medium	0.77
7	Brown	Fine	0.84
9	Auburn	Medium	1.11
40	Dark brown	Fine	2.17
28	Jet black	Thick	0.17 (?)
27	Light brown	Fine	1.30
70	Black and white	Thick	0.56
Average			1.3

Storage for three months does not cause any decrease in niacin content.

As for riboflavin and pyridoxine, storage causes a loss of sixty and eighty per cent of the initial content, respectively, after five months of storage for riboflavin and seven months for pyridoxine. The content of these vitamins in hair from pellagrous Yugoslav subjects reflected an increase rather than a decrease.

After three months of storage, the free pantothenic acid in the hair from control subjects decreased by about one fourth. In hair from pellagrous subjects in comparison, the content was not found lower.

#### REFERENCES

1. SNELL, E. E. and WRIGHT, L. D., *J. Biol. Chem.*, 139: 675, 1941.
2. SNELL, E. E. and STRONG, F. M. *Ind. Eng. Chem. Anal.*, 2nd ed., p. 346, 1939.
3. ATKIN, L., SCHULTZ, A. S., WILLIAMS, W. L. and FREY, C. N. *Ind. Eng. Chem. Anal.*, 15th ed., p. 141, 1943.
4. SKEGGS, H. R. and WRIGHT, L. D. *J. Biol. Chem.*, 156: 21, 1944.
5. STANGL, E. *Internat. Ztschr. Vitaminforsch.*, 24: 9, 1952.
6. GOUNELLE, H. and RICHET, F. *Compt. rend. Soc. de biol.*, 150: 12, 1956.