14830

# The $F_2$ Excretion of Normal Men on Different Levels of Niacin Intake.*

OLAF MICKELSEN AND LESTER L. ERICKSON. (Introduced by Ancel Keys.)
*From the Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis.*

Najjar and his collaborators[1,2] reported the presence in urine from normal individuals of a fluorescent substance which they called $F_2$. This compound was excreted in fairly large amounts when the niacin intake was high. In cases of pellagra the excretion of $F_2$ was diminished or even zero in some cases. When large amounts of niacin were ingested by normal individuals or during the course of pellagra therapy, the $F_2$ excretion increased. Their evidence indicated that this fluorescent substance was related to the metabolism of nicotinic acid.

Recently both the Johns Hopkins group[3,4] and Huff and Perlzweig[5] have shown by means of chemical isolation that the fluorescent substance is a derivative of niacin, suggesting that $F_2$ is N-methylnicotinamide or some compound closely related to it.

A description of the method for the determination of this substance was published by Najjar and coworkers.[6] Later a modified procedure was proposed by Huff and Perlzweig.[7] Recently Najjar published another modification.[8] Some of our results with these procedures have been reported in a preliminary note.[9]

*Experimental.* As a part of a comprehensive study of the B vitamins[10] 4 normal young men were maintained for a period of 161 days on a diet which supplied an average of 12.4 mg of niacin per day. During the same period 4 other men were given the same diet plus an additional 10 mg of niacin. This was followed by a period of 32 days when the niacin intake in the diet was reduced to 0.12 mg per day. The niacin in the diet was determined by the microbiological method.[11] Twenty-four-hour urine samples were collected throughout the period at intervals of several days. These samples were preserved by adding 5 ml glacial acetic acid and 5 ml toluol to the collection bottles. In most cases the analyses were made on the same day the collection was completed.

Throughout the first dietary period, the urines were analyzed by the original method of Najjar, *et al.*[6] This method was slightly modified to permit the determination of thiamine and $F_2$ on the same eluate from the zeolite column. When the severely restricted diet was started, the method of Huff and Perlzweig[7] was used. Najjar informed us of his modified procedure[8] after all of these analyses were completed.

The procedures of Najjar *et al.*[6] and Najjar[8]

* This work was supported in part under the terms of a contract between the Regents of the University and the Office of Scientific Research and Development. Important financial assistance was also provided by the Nutrition Foundation, Inc., the U. S. Cane Sugar Refiners' Association, N.Y., the Corn Industries Research Foundation, N.Y., Swift and Co., Chicago, the National Confectioners' Association, the National Dairy Council, Chicago, and the Graduate Medical Research Fund, University of Minnesota. Merck and Co., Inc., provided a generous supply of pure vitamins.

1 Najjar, V. A., and Wood, R. W., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 386.

2 Najjar, V. A., and Holt, L. E., Jr., *Science*, 1941, **93**, 20.

3 Najjar, V. A., Scott, D. B. M., and Holt, L. E., Jr., *Science*, 1943, **97**, 537.

4 Najjar, V. A., and White, V., *Science*, 1944, **99**, 284.

5 Huff, J. W., and Perlzweig, W. A., *Science*, 1943, **97**, 538.

6 Najjar, V. A., Stein, H. J., Holt, L. E., Jr., and Kabler, C. A., *J. Clin. Invest.*, 1942, **21**, 263.

7 Huff, J. W., and Perlzweig, W. A., *J. Biol. Chem.*, 1943, **150**, 483.

8 Najjar, V. A., *Bull. Johns Hopkins Hosp.*, 1944, **74**, 392.

9 Mickelsen, O., *Federation Proc.*, 1944, **3**, 61.

10 Keys, A., Henschel, A., Taylor, H. L., Mickelsen, O., and Brozek, J., 1944, in press.

11 Snell, E. E., and Wright, L. D., *J. Biol. Chem.*, 1941, **139**, 675.

TABLE I.
Comparison of $F_2$ Excretions by the Old[6] and New Methods[8] of Najjar.
All values expressed in mg N-methylnicotinamide chloride per 100 cc urine and as percentages of the means for the corresponding methods.

| Subject | Old method | | New method | |
|---|---|---|---|---|
| | mg | % of mean | mg | % of mean |
| Wa | 0.378 | 45.5 | 0.252 | 40.1 |
| Jo | 0.489 | 58.9 | 0.318 | 50.6 |
| Gl | 0.567 | 68.3 | 0.441 | 70.1 |
| Se | 0.630 | 75.9 | 0.511 | 81.2 |
| Ja | 0.674 | 81.2 | 0.584 | 92.8 |
| Ph | 0.882 | 106.3 | 0.696 | 110.6 |
| Oa | 1.45 | 174.7 | 1.03 | 163.8 |
| Ol | 1.57 | 189.2 | 1.20 | 190.8 |
| Mean | 0.830 | 100.0 | 0.629 | 100.0 |

were compared with the results shown in Table I. The values in all of our determinations were originally calculated as quinine sulfate equivalents and were then converted to mg of N-methylnicotinamide chloride by multiplying by 35 as suggested by Najjar.[8] The absolute values for $F_2$ excretion as determined by the two methods differ considerably, but in spite of the wide range, the relative ranking of the men was the same in both cases. For each subject, the differences between the percent of the means is within the experimental error of the methods. Although there are not as many comparisons as would be desired, it is believed they indicate that any marked alterations in $F_2$ excretion will show up by either one of the methods. Najjar,[8] furthermore, has indicated that the value of his modified procedure resides primarily in overcoming the false zero $F_2$ excretions on very low niacin intakes and the masking of small amounts of $F_2$ in fasting one-hour or in casual specimens.

*Results.* At the start of the controlled dietary intake of niacin, $F_2$ analyses were made on the 24-hour urine samples collected once each week. Some representative values of the $F_2$ excretion during this period are given in Table II. After the subjects had been on their respective diets for 3 months, a consideration of the results (Table II) indicated that the method of Najjar *et al.*[6] did not differentiate between the 2 levels of niacin intake. On a number of occasions, even negative values were secured in both groups for the urinary excretion of $F_2$. These considerations and others led us to abandon this procedure.

The average for the $F_2$ excretion of the men on the intake of 12.4 mg was 3.22 mg (as N-methylnicotinamide) per day whereas that for the supplemented group was 3.83 mg. There is, however, such great overlapping of the two groups that no acceptable differentiation of the subjects can be made. The averages of all the analyses that were made during this complete period show essentially the same random distribution of the subjects as in Table II.

When the subjects were put on the severely restricted diet, 2 men from the supplemented group and 2 from the restricted group were put on an intake of 0.12 mg niacin while the other 4 were given an additional 10 mg. During this time the urines were analyzed for $F_2$ by the method of Huff and Perlzweig.[7]

Table III shows the results of the urinary excretion of $F_2$ of these subjects during the severely restricted regimen. The values are expressed as mg of quinine sulfate since no N-methylnicotinamide was available when the analyses were made. The results indicate that even when the intake of niacin was restricted to 0.12 mg per day for a period of 32 days, the excretion of $F_2$ showed only a slight and inconsistent decrease as determined by this method. At the end of the period the subjects cannot be divided into their proper groups on the basis of either single excretions or the averages for the entire period.

*Discussion.* A compound as stable as niacin is not likely to be entirely destroyed by the body but at present it appears that the sum of all recognized end products of niacin metabolism in the urine accounts for no more than 10% of the intake.[7-9] Such evidence as is available from related compounds suggests that the excess of niacin intake over metabolic use may be excreted in a conjugated form. It would seem reasonable to suppose that some compound such as $F_2$ might be excreted in proportion to the excess of intake over need or, inversely, to the extent of deficiency. The present evidence indicates that this simple

TABLE II.

Excretion of $F_2$ on Controlled Intakes of Niacin.

24-hour urinary excretion of $F_2$ expressed as mg N-methylnicotinamide chloride. Only a few representative values are given. The controlled diet was started 6-7-43. The method of Najjar *et al.*[6] was used in these analyses.

| Niacin intake | 12.4 mg per day | | | | | 22.4 mg per day | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Subject | Ge | Wi | Tr | Wa | Mean | Sc | No | Jo | Ja | Mean |
| 6-24 | 1.49 | 2.81 | 4.20 | 1.68 | 2.55 | 5.30 | 3.19 | 0 | 2.19 | 3.56 |
| 7-19 | 5.48 | 3.01 | 3.52 | 3.00 | 3.75 | 4.57 | 2.54 | 3.33 | 5.01 | 3.88 |
| 7-26 | 2.89 | 1.51 | 2.95 | 3.06 | 2.60 | 1.88 | 2.91 | 1.69 | 4.88 | 2.84 |
| 8-17 | 1.97 | 1.82 | 4.46 | 4.78 | 3.26 | 5.64 | 5.92 | 4.76 | 6.00 | 5.58 |
| 8-30 | 4.74 | 5.29 | 3.18 | 2.53 | 3.93 | 4.66 | 4.10 | 2.63 | 1.82 | 3.30 |
| Mean | 3.31 | 2.89 | 3.66 | 3.01 | 3.22 | 4.41 | 3.73 | 2.48 | 3.98 | 3.83 |

TABLE III.

Excretion of $F_2$ on Very Low Intakes of Niacin.

24-hour urinary excretion of $F_2$ expressed as mg of quinine sulfate. All analyses were made by the method of Huff and Perlzweig.[7] The diet was started November 15 and continued through December 17, 1943.

| Niacin intake | 0.12 mg per day | | | | | 10.12 mg per day | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Subject | Ge | Wi | Sc | Ja | Mean | No | Jo | Tr | Wa | Mean |
| 11-8 | 0.11 | 0.46 | 0.69 | 1.23 | 0.62 | 0.29 | 0.84 | 0.46 | 0.87 | 0.62 |
| 11-15 | 1.11 | 0.64 | 0.70 | 0.96 | 0.85 | 0.52 | 1.56 | 1.09 | 1.02 | 1.05 |
| 11-18 | 0.26 | 0.16 | 0.30 | 0.26 | 0.25 | 0.24 | 0.35 | 0.41 | 0.26 | 0.32 |
| 11-21 | 0.22 | 0.12 | 0.16 | 0.20 | 0.18 | 0.32 | 0.32 | 0.22 | 0.20 | 0.27 |
| 12-1 | 0.25 | 0.11 | 0.14 | 0.25 | 0.19 | 0.21 | 0.33 | 0.44 | 0.25 | 0.31 |
| 12-7 | 0.69 | 0.70 | 0.24 | — | 0.54 | 0.18 | 0.25 | 0.42 | 0.36 | 0.30 |
| 12-16 | 0.22 | 0.32 | 0.15 | — | 0.23 | 0.26 | 0.35 | 0.33 | 0.44 | 0.35 |
| Mean | 0.41 | 0.36 | 0.34 | 0.58 | 0.41 | 0.29 | 0.57 | 0.48 | 0.49 | 0.46 |

theory does not apply to the rather considerable range of conditions represented in our experiments.

Several possible explanations for the results may be suggested. In the first place the body may require less than 12 mg of niacin per day and all the excess may be eliminated in some form other than $F_2$ or N-methylnicotinamide. No differentiation would then be expected between 12.4 and 22.4 mg per day but it might still be possible to distinguish between lower levels of intake by measurement of $F_2$ excretion. Secondly, the metabolic turnover in the body may be so slow that even a substantially zero intake for 32 days would not be reflected in the excretion of substances like $F_2$. We may note that at no time were there any signs or symptoms in these subjects which could clearly be referred to as a niacin deficiency. Finally, it is conceivable that the particular type of diet used may have altered the bacterial flora of the intestine so that synthesis of niacin from this source obscured the effects of the alterations in intake of niacin. The diet used in the prolonged experiment with niacin at 12.4 and 22.4 mg per day was not remarkable except for a low level of the whole B complex but the diet used for the final 32 days was chiefly of the synthetic type and therefore greatly abnormal.

In any case it appears that measurement of the excretion of $F_2$, under the conditions applied here, is worthless as a means of evaluating the state of niacin nutrition though it may have value in cases of much more prolonged restriction in intake. Both the stability of niacin and the failure of present methods to account for its disappearance after ingestion argue for continuation of the search for the end products. It may be significant that the excretion of $F_2$ shows great variations from day to day, even on a rigidly constant diet and with maintenance of standardized physiological conditions including physical activity. These variations are not reflections of careless technic but continue in spite of

elaborate efforts to control the methods.

We have had some indication that part of the fluorescence measured as $F_2$ is related to thiamine; the blank in the thiamine determination, using zeolite adsorption as in the Najjar method for $F_2$, increases as the thiamine excretion decreases and this is true even though the niacin intake is constant at about 20 mg per day. Time has not permitted us to investigate this question further.

*Summary.* $F_2$ analyses have been made on the urine of normal young men who were maintained on controlled niacin intakes. The method of Najjar showed no difference in the $F_2$ excretion of 2 groups whose intakes over a period of 161 days averaged 12.4 and 22.4 mg of niacin per day. There was no difference in the $F_2$ excretion of 2 groups maintained for 32 days on intakes of 0.12 and 10.12 mg of niacin per day when the method of Huff and Perlzweig was used for the determination.

14831

## Experiments on Erythrocyte Sedimentation Rate*

KARL MEYER, ELEANOR HAHNEL, AND ROSE R. FEINER.

*From the Department of Ophthalmology, College of Physicians and Surgeons, and the Institute of Ophthalmology, Presbyterian Hospital, New York.*

In general the rate of erythrocyte sedimentation (E.S.R.) is considered to be dependent on the nature and concentration of the protein components of the plasma (*lit.*[1]). However there is some experimental evidence suggesting that E.S.R. may likewise be profoundly influenced by non-protein substances. Fahraeus[2] demonstrated that gum arabic added to normal blood caused an increased E.S.R. The chemically closely related type III pneumococcus polysaccharide[3] was shown to have a similar effect.[4] Shedlovsky and Scudder several years ago in an unpublished lecture reported an increase in E.S.R. on the addition of viscous extracts of certain sarcomata which presumably contained hyaluronic acid. Similar experiments were performed by Kendall (personal communication) and by us (unpublished) with pure hyaluronic acid.

In this paper experiments will be presented which, in combination with published evidence, indicate that probably all highly asymmetrical molecules of large size cause an increase of E.S.R. when added to normal blood. Experiments on the intravenous injection of hyaluronate and hyaluronidase are likewise reported.

*Experimental.* The first experiments deal with the *in vitro* increase of E.S.R. induced in normal human blood by various substances. E.S.R. in all experiments was measured by the Westergren method, making no correction for cell volume or protein concentration. All blood samples were obtained from healthy young males and, after mixing with the additions indicated in the experiments, were incubated for 30 min. at 37°C. The samples were then drawn up in the sedimentation tubes and read every 15 minutes for 1 hour.

The samples of hyaluronic acid used were prepared from cattle synovial fluid,[5] from a

* This work was supported in part by a grant from the Josiah Macy, Jr., Foundation. Part of these data was reported at the meeting of the American Chemical Society, Biochemical Section, New York, Sept., 1944.

1 Nichols, R. E., *J. Lab. and Clin. Med.*, 1942, **27**, 1317.

2 Fahraeus, R., *Acta Med. Scand.*, 1921, **55**, 3; *Physiol. Rev.*, 1929, **9**, 241.

3 Heidelberger, M., Avery, O. T., and Goebel, W. F., *J. Exp. Med.*, 1929, **49**, 847.

4 Nungester, W. J., and Klein, L. F., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 315.

5 Meyer, K., Smyth, E. M., and Dawson, M. H., *J. Biol. Chem.*, 1939, **128**, 319.