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Research Article

F_1 AND F_2 OF NAJJAR AND HOLT IN THE URINE OF NORMAL YOUNG MEN [1]

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Najjar and Holt (1, 2) have demonstrated that their fluorescent urinary pigments, F_1 and F_2, are related to nicotinic acid metabolism in such a way that their estimation in urine is of diagnostic use in human pellagra (1) and in canine black tongue (1, 2). As nicotinic acid deficiency progresses, the daily excretion of F_1, starting from a low level, slowly rises and then falls again; and the daily excretion of F_2, starting from a normal level, falls progressively to zero. Vigorous treatment of pellagrins with nicotinamide causes, within 3 days, a large increase in F_2 and a decrease in F_1. Extending this work, Holt and Najjar (3, 4, 5) proposed the routine measurement of urinary F_2 in nutritional surveys as an indication of the nicotinic acid content of the body. They state that when F_2 is absent in urine collected in the post-absorptive state,[3] the patient's stores of nicotinic acid are low. Oral administration of nicotinamide to normal subjects in a good nutritional state leads to a prompt increase in F_2, maximal in 2 to 4 hours and subsiding to normal within 6 hours. Coulson and his coworkers (6) have also made a detailed study of the effects of various nicotinic acid derivatives on urinary F_2 estimations. Their results are much the same as those of Najjar and Holt.

In developing suitable rapid field methods for assessing the nutritional status of young men (7), we have investigated the conditions under which the estimation of urinary F_1 and F_2 might be expected to yield useful information. In particular, we sought to answer 3 specific questions: first, whether the level of these pigments in urine in a post-absorptive state agrees with the criteria of Holt (3); second, whether their excretion following administration of nicotinamide correlates with other clinical data; and third, whether their excretion following the administration of various mixtures of vitamins has the same significance as after nicotinamide alone. Our observations have been limited solely to normal young men, and we have not studied pellagrins.

EXPERIMENTAL

We will discuss first, analytical methods; second, the stability of F_2 in urine; and third, the F_1 and F_2 elimination of young men under a variety of conditions.

Analytical methods

All estimations of F_1 and F_2 reported in this paper were carried out by the method of Najjar and Wood (8) or by a modification of this method for rapid field use to be described in a forthcoming paper (7). This modification is different from the method of Huff and Perlzweig (9). In our determination, fluorometry was either photoelectric or visual and the reference standards were either quinine or thiochrome.

Stability of F_2 in urine

Field surveys sometimes necessitate the collection of specimens in climatic extremes, transportation over considerable distances, and storage for various lengths of time before analysis. The stability of F_2 under simulated field conditions was therefore investigated. Random samples of urine were collected from several normal

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[3] The term "post-absorptive" is used in the sense of Benedict. A subject is in the post-absorptive state when he is resting 14 hours or more after his evening meal and after a night's sleep. In other words, he is fasting before breakfast.

men and from several men who had taken orally 100 mgm. of nicotinamide, 12 hours before collection. In each case, the specimens were pooled, acidified to approximately pH 4 with glacial acetic acid, and some portions were stored in an ice-box at 10° C. and others in an incubator at 50° C.

Table I summarizes a typical experiment. (Throughout this paper 3 arbitrary conventions in terminology will be followed: first, the expression "γ" represents the amount of F_1 or F_2 in quinine units; second, the term "normal urine" refers to urine collected from men on a good normal diet, not supplemented by vitamin preparations; and third, the term "loaded urine" refers to urine collected from men who had within the previous 12 hours ingested one or another mixture of vitamins.) This table brings out 4 points. First, within 6 days, F_2 in normal urine showed no significant change at either temperature. Second, within 2 days of storage, F_2 in loaded urine increased significantly at both temperatures, the increase continuing for at least 6 days. Third, the increase in F_2 in loaded urine was not affected by changes of temperature. Fourth, the increase was not prevented by thymol, indicating that bacterial growth was not the cause of the increase.

The stability of F_2 has been discussed by Najjar and Wood (8), who found that the pigment is destroyed slowly in air and more rapidly in the presence of alkali and potassium ferricyanide. In the light of the recent studies on the relation between F_2 and N-methylnicotinamide (10 to 13), a reasonable hypothesis to explain our results is that in normal urine no precursor of N-methylnicotinamide is present, but in loaded urine, such a precursor is present in significant amounts and breaks down by some mechanism other than bacterial action. The practical conclusions of our studies on stability are: first, insofar as F_2 is concerned, normal urine may be transported safely, stored, and analyzed at leisure, provided the pH is lower than 4; and second, that loaded urine should be handled reasonably promptly.

TABLE I

Stability of F_2 in urine

Storage tempera-	Sample	Days exposed			
		0	2	4	6
°C.		γ per cent			
10	Normal urine	1	1	1	1
10	Loaded urine	14	22	19	28
10	Loaded urine + thymol	14	24	19	31
50	Normal urine	1	1	1	1
50	Loaded urine	14	24	24	31
50	Loaded urine + thymol	14	31	24	31

F_1 and F_2 elimination under a variety of conditions

In the succeeding paragraphs, 4 points will be brought out. First, with certain important reservations, our findings substantiate in general the statements made by Holt (3) concerning the significance of F_2 in post-absorptive urine. Second, responses to identical oral doses of nicotinamide vary among subjects. This variability tends to invalidate the use of F_2 alone as a criterion in nicotinamide tolerance tests. Third, F_1 excretion is probably influenced by the dietary level of thiamine. Finally, as would be expected from points 2 and 3, the interpretation of the changes in F_1 and F_2 after the administration of various mixtures of vitamins is much complicated by individual idiosyncracies in the metabolism of nicotinic acid and also by the level of vitamins in the subject's diet.

(1) F_1 and F_2 in post-absorptive urine. Samples of urine were obtained over 1- to 3-hour periods from 169 normal young men in the post-absorptive state. Of these, 20 were subsisting in New England on an adequate civilian diet and 149 were existing in the Mojave Desert on a diet containing over 100 grams of protein daily, with egg, milk, and meat products at every meal. In none of these 169 men were any signs or symptoms of nicotinic acid deficiency seen.

In the 169 specimens of urine, the average hourly excretion of F_1 was 0.2γ, and it was zero in 95 cases (see Table V). Therefore, the presence or absence of F_1 in the fasting urine bears no simple relation to the amount of nicotinic acid in the tissues or in the diet.

The average hourly excretion of F_2 was 2.0γ and in 6 cases was zero (see Table V). These results should be compared with Holt's statement (3): "The quantity of . . . F_2 found in the test specimen indicates the extent of the body reserves of . . . nicotinic acid. As long as any . . . F_2 is found in the test specimen this indicates . . . that deficiency . . . is not to be feared. A zero excretion . . . indicates, however, that . . . such an individual is potentially deficient." In our group of 169 men, whose intake of nicotinic acid was unquestionably adequate, the F_2 excretion in the post-absorptive state was in reasonably good agreement with the dietary and medical data. However, 6 cases were found whose F_2 excretion was zero without any other evidence of nicotinic acid deficiency. Holt's criterion, therefore, is possibly too strict, and before a diagnosis of early nicotinic acid deficiency can be made, other dietary and medical evidences should substantiate a zero F_2 in the post-absorptive urine.

In contrast to the above findings on normal young men, we found in one case that the presence of F_2 in considerable amounts is compatible, at least for short periods of time, with complete nutritional deficiency. Table II contains data on this one man. During 16 days of voluntary fasting, no F_1 appeared, riboflavin fluctuated erratically, thiamine and ascorbic acid decreased steadily, and F_2 increased tenfold. In this case, the levels of thiamine and vitamin C changed with the patient's known nutritional state, but the F_2, if considered alone, would have led to the fallacious conclusion that he was well supplied with nicotinic acid.

TABLE II

Changes in urinary vitamins during a voluntary complete fast

Period of fast	Substance in urine				
	F_1	F_2	B_1*	B_2**	C***
day	*γ per 24 hours*		*γ per 24 hours*		*mgm. per 24 hours*
1	0	3	104	1040	
4	0	2	83	990	75
6	0	30	74	1225	44
8	0	34	66	1753	0
16	0	30	25	990	0

* Analysis by method of Egaña and Meiklejohn (14).
** Analysis by method of Najjar (15).
*** Analysis by method of Mindlin and Butler (16).

(2) *Individual responses to oral doses of nicotinamide.* During an investigation of the reliability of the oral nicotinamide tolerance test, we found that individual responses were varied. This variability is illustrated in Table III. Three normal young men, subsisting on adequate diets, received 50 mgm. of nicotinamide orally at each meal during a period of 2 or 3 days. F_2 was estimated in specimens of urine collected in the post-absorptive state as well as during the rest of the day. The table shows 2 points. First, one subject, P. R., showed a large response in both specimens of urine; another, F. C., no response; and third, F. S., a moderate response. Second, in one case, P. R., the fasting hour specimen showed the greater response, and in another, F. S., the 24-hour specimen. It is concluded that this wide individual variation among men known to be adequately supplied with nicotinic acid indicated that F_2 alone cannot be used as an accurate estimate of the effects of ingestion of nicotinamide.

TABLE III

Individual responses to oral doses of nicotinamide

Subject	Period	F_2 in urine, Average 2 hour fasting	Total in 24 hours
		γ per hour	
P.R.	Before	12	12
	2 days' loading*	126	28
	After	2	
F.C.	Before	8	8
	3 days' loading*	6	6
	After	9	4
F.S.	Before	5	3
	3 days' loading*	7	14
	After	4	

* All subjects took 50 mgm. of nicotinamide by mouth at each meal.

(3) *Effect of dietary level of thiamine on F_1 and F_2 excretion.* In tolerance tests employing mixtures of vitamins, we frequently found large amounts of F_1 in the urine. The effect of thiamine on the excretion of F_1 and F_2 was therefore investigated. The same 3 subjects described in the preceding paragraph ingested 5 mgm. of thiamine hydrochloride at each meal for 3 days. Specimens of urine were collected as in the nicotinamide tolerance tests described above. Table IV shows the results. Particular attention may be called to 2 points. First, in 1 subject, P. R., there was a significant increase in F_1 following thiamine ingestion, and in the other 2, there was

no significant increase. All subjects showed a small increase in F_2. It is concluded from these observations that in some individuals, the interpretation of urinary levels of F_1 and F_2 may be complicated by this possible effect of thiamine.

TABLE IV

Individual responses of F_1 and F_2 in mgm. per hour following ingestion of thiamine

Subject	Period	Average F_1 2-hour fasting	Average F_1 Total 24 hours	Average F_2 2 hour fasting	Average F_2 Total 24 hours
		γ per hour		*γ per hour*	
P. R.	Preloading	5	2	7	3
	2 days' loading*	12	39	7	9
	Post-loading	13	7	12	12
F. C.	Preloading	12	13	4	4
	3 days' loading*	6	11	6	10
	Post-loading	16	9	8	8
F. S.	Preloading	0	0	5	4
	3 days' loading*	0	0	6	7
	Post-loading	0	0	5	3

* All subjects took 5 mgm. thiamine hydrochloride at each meal.

(4) F_1 and F_2 excretion following ingestion of various mixtures of vitamins. In field surveys of nutritional status, it is common to perform tolerance tests with mixtures of vitamins. In the course of running such tests, we have obtained data following single administrations, and following administrations daily for 8 weeks.

Table V shows the variability in response of F_1 and F_2 among the 169 men described above whose nicotinic acid intake was unquestionably adequate. We have previously discussed F_1 and F_2 in their post-absorptive urine. We shall now consider F_1 and F_2 separately in the case of their loaded urine.

An increase in F_1 following the test dose was seen in about 10 per cent of the subjects. This increase was possibly due to thiamine as described above. We may conclude that in field surveys little information is to be gained by estimating F_1 in tolerance tests.

An average hourly increase of about tenfold was seen in the F_2 elimination following the test dose. Nevertheless, about 15 per cent of the men failed to respond. We draw 2 conclusions with respect to F_2 in tolerance tests. First, a high F_2 following the test dose is significant and probably means adequate saturation of the body with nicotinic acid. Second, a low F_2 must be interpreted with caution with due allowances for variability and with careful consideration of other dietary and medical data.

Very considerable increases in F_1 and F_2 may be observed following ingestion of large doses of vitamins over prolonged periods. In Table VI, urinary data are presented for groups of subjects subsisting for 8 weeks on normal diets, on diets low and high in protein, and on diets devoid of ascorbic acid. All subjects received a daily supplement of brewers' yeast extract,[4] containing in each dose 5 mgm. of thiamine, 2 mgm. of ribo-

[4] Standard Brands, Inc., kindly donated the Type III extract of brewers yeast which we used in part of this study.

TABLE V

Excretion of F_1 and F_2 before and during 4 hours following oral doses of vitamins *

Group of subjects	Number of subjects		F_1 Fasting urine	F_1 Total excess in 4 hours	F_2 Fasting urine	F_2 Total excess in 4 hours
			γ per hour		*γ per hour*	
New England	20	Mean	0.1	1	1.2	75
		Range	0 to 0.7	0 to 9	0 to 3	4 to 221
	Percentage of men showing increase**			10		100
Mojave Desert	149	Mean	0.3	2.3	2.4	58
		Range	0 to 3	0 to 136	0 to 20	0 to 844
	Percentage of men showing increase**			8		81

* The test dose was an aqueous solution containing 5 mgm. thiamine hydrochloride, 5 mgm. riboflavin, 50 mgm. nicotinamide, and 500 mgm. ascorbic acid.

** "Percentage showing increase" means the percentage of subjects who excreted more F_1 and F_2 per hour following the dose than before.

TABLE VI

*Excretion of F_1 and F_2 after supplementation with yeast for 8 weeks**

Type of diet	Number of subjects	F_1 Mean	F_1 Range	F_2 Mean	F_2 Range
		γ per day		*γ per day*	
Normal	7	21	0 to 60	27	7 to 39
Low protein	6	15	0 to 40	33	7 to 56
High protein	8	32	0 to 74	34	1 to 55
Vit. C free	14	21	0 to 88	33	10 to 61

* Each subject received one daily dose of Fleischman's Type III Brewers' Yeast Extract containing 5 mgm. thiamine, 2 mgm. riboflavin, and 11.5 mgm. of niacin.

flavin, and 11.5 mgm. of niacin. The average excretion of F_1 and F_2 per day was high in almost every case, the average F_2 excretion being the same as that of the fasting man after 6 days. These results emphasize again the necessity of interpreting urinary data after consideration of other clinical data.

COMMENT

In considering the practical utility of estimating F_1 and F_2 in nutritional surveys, the 2 substances should be discussed separately.

The presence or absence of F_1 in the urine of normal subjects, either in the post-absorptive state or after test doses of vitamin, appears to bear little, if any, relation to their nutritional state. We must emphasize that this conclusion does not necessarily apply to pellagrins.

The interpretation of the levels of F_2 in the urine is complicated by the wide variety of individual responses to the dietary level of nicotinic acid. Several possible causes for this variability present themselves at once, some more likely than others. First, individual idiosyncrasies of absorption, although possible, appear to be unlikely as the chief cause of variation. In our series, wide individual variations were present even after 8 weeks of supplementation of diets already rich in animal protein. Equally wide variations were found after only 2 or 3 days of supplementation. Second, different responses at different stages of chemical unsaturation may be responsible for a certain percentage of cases showing anomalous responses. This phenomenon has been shown by Najjar and Holt (1, 2) in canine black tongue and in human pellagra and appeared to be operative in our subject who fasted for 16 days. This cause can hardly play an important rôle in young men on normal diets. Third, the most reasonable hypothesis seems to be that the end-products of nicotinic acid metabolism occur in urine in many forms and in different proportions in different subjects. Ingested nicotinamide is known to give rise in the urine to nicotinic acid, nicotinamide, nicotinuric acid, and N-methylnicotinamide (the precursor of F_2) (17). For unexplained reasons, one or the other of these substances sometimes predominates, and some may not appear at all (17). Convincing data on this multiplicity and variability of nicotinic acid intermediaries have been presented by Sarett, Huff, and Perlzweig (17). One mechanism has been elucidated by Perlzweig and coworkers (18), who showed that the aerobic methylation of nicotinamide by slices of rat liver is sometimes, but not always, enhanced by methionine.

In view of evidence such as the above, it would appear desirable to estimate as many as possible of the urinary nicotinic acid derivatives in assessing the status of the body's stores of the vitamin. At present, this is a laborious task, even in a well-equipped laboratory. For field surveys on normal young men, it appears that F_2 is the easiest and quickest single substance to assay. Data on it must, however, be interpreted with caution, after careful correlation with other independent evidence and with full realization of the limitations of the findings.

SUMMARY

1. An investigation has been made into the utility of routine estimations of the fluorescent urinary pigments, F_1 and F_2 of Najjar and Holt, in nutritional surveys of normal young men.

2. F_2 in specimens of urine obtained in the post-absorptive state is stable for at least a week at 50° C. Under the same conditions, F_2 in urine after an oral dose of nicotinamide increases independently of bacterial action.

3. The following conclusions are drawn concerning the urinary level of F_1: (*a*) the presence or absence of F_1 in the urine of normal young men, either in the post-absorptive state or after test doses of vitamins, bears little or no relation

to nutritional state; (*b*) in some subjects, it was discovered that ingestion of thiamine appears to increase the excretion of F_1.

4. The following conclusions are drawn concerning the urinary level of F_2 in the post-absorptive state: (*a*) the level of F_2 in the urine of normal young men correlates reasonably well with other dietary and clinical evidence concerning their nicotinic acid stores; (*b*) however, a small percentage of men known to be eating a diet adequate in nicotinic acid normally excrete no F_2 in the urine; and (*c*) the urinary F_2 of a man who fasted for 16 days reached high levels.

5. The following conclusions are drawn concerning the level of F_2 in urine after test doses of vitamins: (*a*) the usual response of normal men to test doses of nicotinamide is to increase the excretion of F_2; (*b*) a small percentage of men known to be adequately supplied with dietary nicotinic acid shows no increase in F_2 after test doses; (*c*) the level of F_2 must be interpreted with caution, with due allowance for variability and careful consideration of other dietary and medical data.

6. A reasonable explanation for the extreme variability of urinary F_2 is presented. There is known to be a multiplicity of urinary intermediaries in nicotinic acid metabolism, of which F_2 is only one. For unexplained reasons, one or the other may predominate at the expense of the rest, and some may not appear at all after test doses of nicotinamide. Hence, as many as possible of these intermediaries should be estimated when nicotinic acid stores are being assessed.

6. For field studies, F_2 is the easiest derivative of nicotinic acid to estimate. In our experience, its estimation in post-absorptive urine and in the urine following test doses of nicotinamide yields useful information. However, caution must be used in interpretation.

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