JCI The Journal of Clinical Investigation

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J Clin Invest. 1942;21(3):263-267. https://doi.org/10.1172/JCI101298.

Research Article

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THE EXCRETION OF SPECIFIC FLUORESCENT SUBSTANCES IN THE URINE IN EXPERIMENTAL NICOTINIC ACID DEFICIENCY [1, 2]

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(Received for publication November 12, 1941)

In a recent publication (1) the authors have described the presence in urine of certain fluorescent substances which were found to vary in a characteristic way in pellagra. One of these substances, designated F_1, was found in relatively small amounts in normal urine but in larger quantities in pellagra. The second substance, designated F_2, developed fluorescence only on the addition of alkali. F_2 was not found in the urine of pellagrins but reappeared after nicotinic acid therapy. The administration of nicotinic acid to normal subjects caused an increased elimination of F_2. These facts are summarized in Table I, which also gives the characteristics of the fluorescent spectra of the two substances with ultraviolet light. Photographs of the fluorescent spectra are shown in Figure 1.

To explain these phenomena it was suggested that F_1 was converted into F_2 through the agency of some nicotinic acid containing enzyme, a deficit of nicotinic acid causing the accumulation of F_1 which could no longer be converted into F_2. It was further suggested that the accumulation of F_1 might be related to the photosensitivity of the skin seen in pellagra.

TABLE I

Features of fluorescent substances as observed in human urine

	F_1	F_2
	Little in normal urine	Much in normal urine
	Abundant in pellagra	Absent in pellagra
Fluorescence	Whitish, violet blue	Greenish blue
Range of fluorescent emission	4000–4800 A°	4200-5400 A°
Maximum emission	4350 A°	4550 A°

Since natural pellagra is seldom an uncomplicated nicotinic acid deficiency (2), it seemed desirable to study the excretion of these fluorescent substances in animals with a pure nicotinic acid deficiency.

EXPERIMENTAL

Four dogs were employed in this study. These animals had previously been used for a nicotinic acid deficiency experiment, but had subsequently received treatment with nicotinic acid. There was no reason to believe that their recovery was not complete at the onset of the present study. Throughout the course of the study the animals received unlimited quantities of a black-tongue diet consisting of:

	per cent
Yellow corn meal	65
Cow peas	8
Purified casein	7.5
Sucrose	7
Cotton seed oil	5
Cod liver oil	5
NaCl	1
$CaCO_3$	1.5

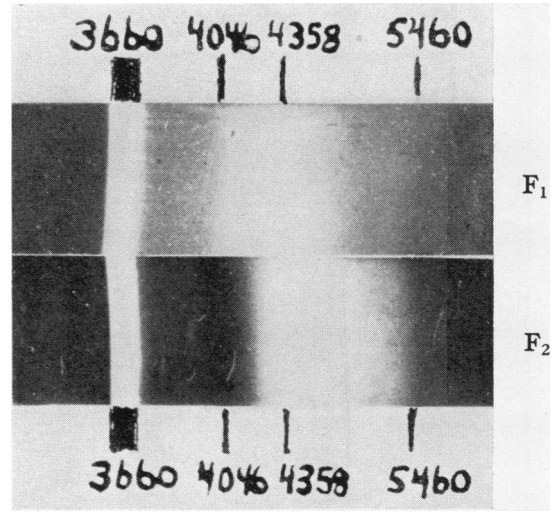

FIG. 1. PHOTOGRAPH OF THE FLUORESCENT SPECTRA

[1] Read before the Society for Pediatric Research, Atlantic City, New Jersey, May 6th, 1941.

[2] Aided by grants from Mead Johnson and Company, Evansville, Indiana, and from the Rockefeller Foundation Fluid Research Fund.

[3] Fleischmann Fellow in Medicine.

In addition, supplements of thiamin and riboflavin were given to each dog twice a week in doses of 0.175 mgm. per kilogram. The dogs were put in metabolism cages every few days in order to obtain 24-hour specimens of urine. The urine was collected in bottles containing glacial acetic acid (3 per cent of the anticipated daily volume of urine) and was assayed for F_1 and F_2 in quinine units by procedures described elsewhere (1, 3).

RESULTS

The course of the F_1 and F_2 excretion in these animals is shown in Figures 2, 3, 4 and 5. It may be noted that in all but one of the animals the F_2 excretion at the start of the experiment exceeded that of F_1. As the experiment continued, F_2 excretion gradually decreased to zero; this occurred more rapidly in the small dog than in the larger animals. The excretion of F_2 remained at the zero level unless nicotinic acid was given. Coincident with the fall of F_2 in the urine, there was a rise in the excretion of F_1, which reached a maximum at or shortly after the time that the F_2 output ceased. This maximum F_1 output was, however, not sustained; as the diet continued, the F_1 showed a gradual decline to

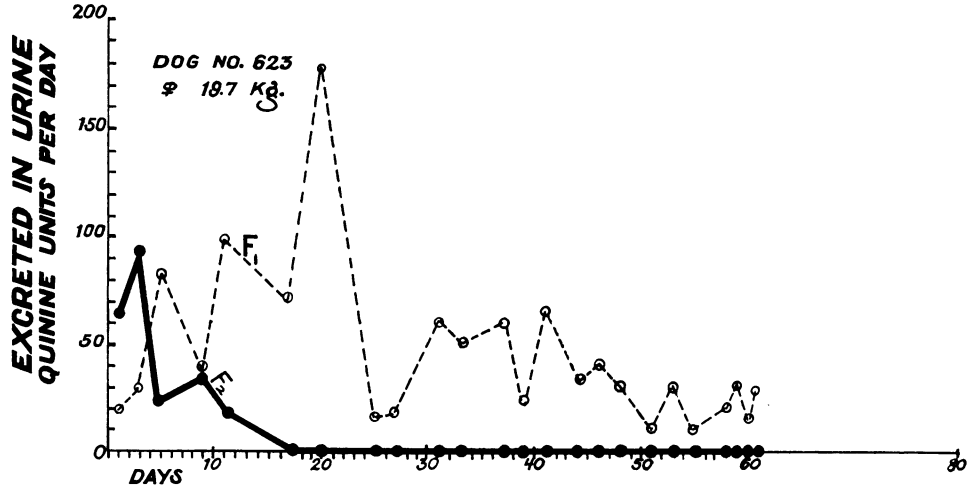

FIG. 2. EXCRETION OF FLUORESCENT SUBSTANCES IN EXPERIMENTAL NICOTINIC ACID DEFICIENCY

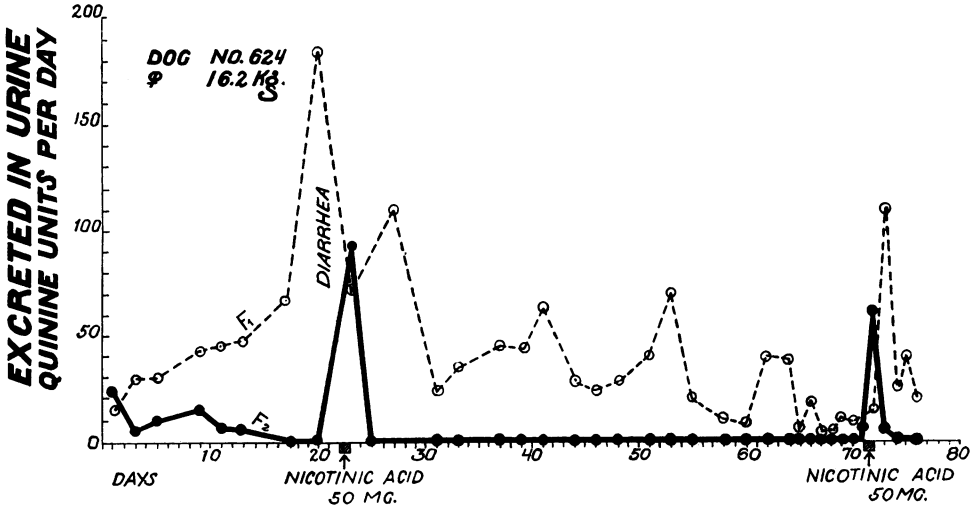

FIG. 3. EXCRETION OF FLUORESCENT SUBSTANCES IN EXPERIMENTAL NICOTINIC ACID DEFICIENCY

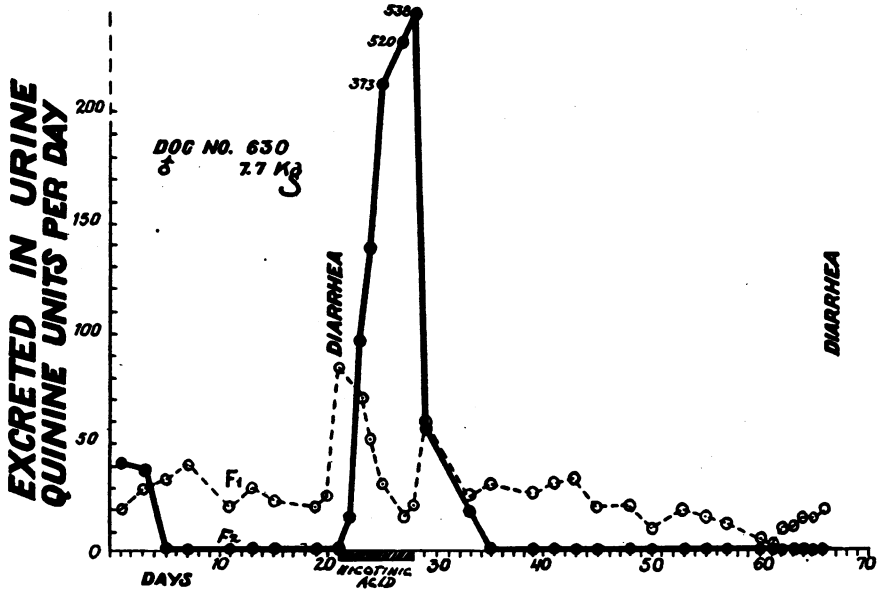

FIG. 4. EXCRETION OF FLUORESCENT SUBSTANCES IN EXPERIMENTAL NICOTINIC ACID DEFICIENCY

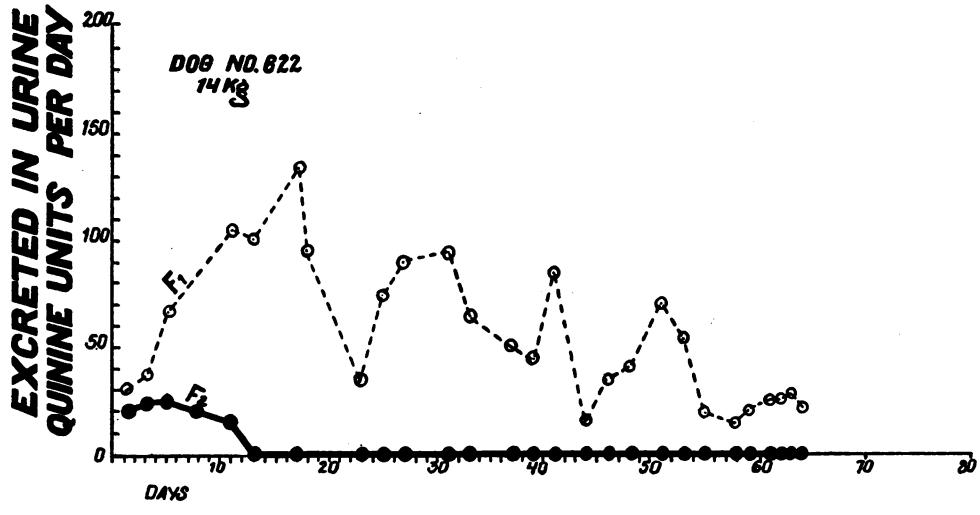

FIG. 5. EXCRETION OF FLUORESCENT SUBSTANCES IN EXPERIMENTAL NICOTINIC ACID DEFICIENCY

values approximating those at the onset of the experiment.

The effect of nicotinic acid therapy is shown in Dogs 624 and 630. The former animal developed diarrhea on the 20th day of the experiment and was subsequently given a single 50 mgm. dose of nicotinic acid orally. This medication caused a cessation of the diarrhea, a prompt rise in F_2 and a diminution in F_1 excretion. The F_2 excretion was shortlived, however. In the next specimen it had again disappeared and F_1 had increased, although the high F_1 excretion was not sustained. On the 71st day of the experiment a second 50 mgm. dose of nicotinic acid was given. This too, was followed by the prompt reappearance of F_2 in the urine. The F_1, however, which had by then reached a low level, did not fall as a result of this dose. Indeed, it may be noted that

it showed no change at all for 24 hours, but following this exhibited a sharp but transitory increase.

The small dog Number 630 showed, in general, a similar response to nicotinic acid. On the 21st day of the experiment he developed diarrhea. His F_1 excretion had by that time risen from 20 to 170 units per day, and his F_2 had fallen to zero. He was then given 50 mgm. nicotinic acid a day by mouth for one week. This resulted in a rise of F_2 to extraordinarily high levels and in a simultaneous decrease of F_1. Following the withdrawal of nicotinic acid, the F_2 excretion fell to zero in the course of a week, and F_1, after a temporary rise, continued to decline steadily until the termination of the experiment.

COMMENT

The observations made on these animals confirm those made on 4 pellagrins studied by us (Table II). The dog experiments, like the studies of patients, demonstrate the absence of F_2 excretion and the tendency of F_1 to rise. Somewhat unexpected, however, was the observation made on the dogs that the rise in F_1 was not indefinitely sustained. In examining the human data from this point of view, it is worthy of note that the most severe case of all, as judged by history, symptoms and reappearance of F_2 in the urine after therapy (Patient E), did not show as high an F_1 excretion as did another definitely milder case (Patient S). It now seems likely that the stage of high F_1 excretion had been passed in this severe case, and that the condition was comparable to that observed in our dogs after many weeks on the experimental diet.

Although, in general, a reciprocal relation between F_1 and F_2 excretion seems to hold, it is now apparent that this relationship is not a perfect one. The fall in F_2 on withdrawing nicotinic acid is not always accompanied by an equally prompt and impressive rise in F_1 (Dog 630). Likewise the administration of nicotinic acid may cause a rise in F_2 excretion far greater than the fall in F_1 (Dog 630) or may under other circumstances (Patient E) cause a reduction in F_1 without any corresponding rise in F_2.

In interpreting these facts, one must bear in mind the possibility that one or both of these substances may be of physiological importance, and that the failure of one to appear quantitatively as the other disappears may be due to a demand made by the body which results in utilization rather than excretion. It is also possible that intermediary non-fluorescent compounds are formed in the conversion of F_1 to F_2. Furthermore, one cannot deny the possibility that the reactions causing the disappearance of F_1 and the appearance of F_2 are two independent reactions, both of which are catalyzed by nicotinic acid.

The gradual decrease in F_1 excretion in the later stages of the deficiency also demands an explanation. In the absence of knowledge of the chemical nature or precursors of this substance, this can hardly be discussed with profit at the present time. It is hoped that studies now in progress will throw some light on the subject.

SUMMARY

The fluorescent substances F_1 and F_2 have been followed in the urine of dogs with experimental

TABLE II

Urinary excretion of F_1 and F_2 in pellagrins *
(Expressed in quinine units)

Subject	Severity of symptoms	Before treatment		After 50 mgm. nicotinic acid by mouth	
		F_1	F_2	F_1	F_2
W.	Very mild	12	0	22	18
H.	Moderately severe	38	0	26	17
S.	Moderately severe	104	0	60	11
E.	Very severe	64	0	40	0
Normal control subjects		10–15	20–35	15–20	35–50

* Figures represent excretion during a 4-hour period.

nicotinic acid deficiency. Observations on these animals are in agreement with those made in human pellagra that acute nicotinic acid deficiency is characterized by the disappearance of F_2 excretion and a rise in F_1 excretion. As the disease becomes more chronic, the excretion of F_1 likewise tends to fall. The effect of nicotinic acid in reversing these changes is illustrated.

BIBLIOGRAPHY

1. Najjar, V. A., and Holt, L. E., Jr., The excretion of specific fluorescent substances in the urine in pellagra. Science, 1941, **93**, 20.
2. Sydenstricker, V. P., Clinical manifestations of nicotinic acid and riboflavin deficiency (pellagra). Ann. Int. Med., 1941, **14**, 1499.
3. Najjar, V. A., and Wood, R. W., Presence of a hitherto unrecognized nicotinic acid derivative in urine. Proc. Soc. Exper. Biol. and Med., 1940, **44**, 386.