A RAPID METHOD OF ESTIMATING N^1-METHYLNICOTINAMIDE IN URINE*

Sirs:

Having established that N^1-methylnicotinamide is the chief excretion product of nicotinic acid metabolism in human urine,[1, 2] we have made a large number of determinations employing a modification of Najjar and Holt's method described elsewhere in this *Journal*.[1] Although the method is quite simple, the time required for the laborious preparation and washing of the zeolite columns limits its usefulness. A number of trials have shown that without any appreciable sacrifice in accuracy the adsorption on and elution from the zeolite columns may be omitted and the fluorescent substance determined directly in butanol extracts of the urine, literally in a few minutes. This direct method is particularly applicable to saturation or tolerance tests after a dose of nicotinic acid or of its amide.

The urines in such tests are best diluted 10-fold with 25 per cent KCl solution. To a 10 ml. aliquot of the diluted urine in a test-tube, made alkaline with 2 or 3 drops of 10 N NaOH solution, 0.5 gm. of Lloyd's reagent is added, the contents mixed for 1 minute by inversion, centrifuged for 2 to 3 minutes, and the supernatant fluid decanted. This procedure was shown to destroy completely the fluorescence of N^1-methylnicotinamide, without affecting the other pigments of urine which are not adsorbed at the alkaline pH. Hence this proved to give the best blank values we have been able to obtain so far. An aliquot of 1 to 5 ml. of the diluted urine containing 3 to 15 γ and simultaneously an equal volume of the blank prepared as above are placed in 125 ml. separatory funnels, extracted with *n*-butanol, and the fluorescence measured exactly as described in our previous article.[1]

In the table are given values for N^1-methylnicotinamide in urines of six patients, collected for 14 hours after an oral dose of 500 mg. of nicotinamide. When compared, the figures obtained by fluorescence analyses directly and after adsorption on and elution from permutit show substantial agreement.

"Trigonelline" values are included for comparison; these were obtained by the older colorimetric method[3] and include both the betaine trigonelline and N^1-methylnicotinamide. These figures and other data on hand indi-

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[1] Huff, J. W., and Perlzweig, W. A., *J. Biol. Chem.*, **150**, 395 (1943).

[2] Sarett, H. P., *J. Biol. Chem.*, **150**, 159 (1943).

[3] Perlzweig, W. A., Levy, E. D., and Sarett, H. P., *J. Biol. Chem.*, **136**, 729 (1940).

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cate that in human urine N^1-methylnicotinamide comprises practically all of the methylated nicotinic acid previously determined and designated as trigonelline. It should also be noted that since trigonelline does not affect the fluorescence measurement, it is not necessary to omit trigonelline-containing foods (coffee, legumes) when nicotinic acid excretion tests are conducted by the above method.

Subject No.	Direct method	Permutit method	"Trigonelline" method
	γ per ml.	*γ per ml.*	*γ per ml.*
1	41	41	47
2	39	40	39
3	40	48	47
4	113	127	117
5	106	109	106
6	45	45	41

The direct method is also applicable to urines of subjects not receiving nicotinic acid other than dietary. Thus in three normal subjects urines collected 2 hours after breakfast gave the following N^1-methylnicotinamide fluorescence values obtained by the direct and permutit methods, in micrograms per ml.: 6.0 and 5.75, 10.6 and 10.0, 6.0 and 5.9, respectively. In urines with a much lower concentration, 0 to 2 γ per ml., the values become quite uncertain, regardless of the method employed, and may be useful only for comparative purposes. Such urines are analyzed directly without dilution.

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