Purified preparations of F_2 more than 100 times as potent in fluorescent units as the original permutit eluates[4] have been obtained from human urine by procedures to be published shortly. The final product is a waxy yellowish brown solid which has been crystallized. It is free from niacin, as judged by a negative immediate cyanogen bromide reaction, but possesses biological properties similar to niacin. One hamster rendered moribund on a niacin-free diet was restored to normal behavior within two hours after oral administration of purified F_2. Purified F_2 is a powerful bacterial growth catalyst for *E. coli* and *H. influenzae*.[5] F_2 occurs in the urine of animals that do not require niacin in their diet (pigs; rats); it occurs in many rat tissues, notably in the liver.

Purified F_2 is readily extracted by butyl alcohols only from alkaline solutions, which explains our observation[6] that alkalinization of urinary eluates was necessary before butyl alcohol extraction to obtain F_2 fluorescence. In acid, neutral or weakly alkaline solutions F_2 exhibits its characteristic fluorescence[7]; with strong alkali fluorescence becomes more greenish. This change is reversible on the addition of acid. On standing with alkali fluorescence gradually fades and a yellowish color develops, changes which are irreversible. F_2 oxidizes slowly in the air, more rapidly in the presence of alkali and $K_3Fe(CN)_6$; the characteristic blue fluorescence is lost and a violet fluorescence develops. This change is irreversible. Addition of acetone to alkaline F_2 solutions produces an intense yellow solution with a green fluorescence resembling that of uranium glass; this change is irreversible. Sulfanilic acid produces an orange red color with loss of fluorescence.

F_2 appears to be a pyridine compound. On alkaline hydrolysis a positive cyanogen bromide test[8] is obtained, not present originally. F_2 is destroyed rapidly by HNO_2.

Since our original communication[4] we have investigated various other pyridine derivatives and wish to report that none of the following can be identified with F_2: cozymase, dihydrocozymase, desaminocozymase, nicotinamide nucleoside and acetyl nicotinamide. We have for some time been familiar with the physical and chemical similarity between F_2 and the N-methyl reduction products of nicotinamide studied by Karrer *et al.*,[9] and had embarked on a program of preparing and testing these compounds when the communication of Huff and Perlzweig was sent to us. Our results to date indicate that one of the dihydro-N-methyl nicotinamides is indistinguishable from F_2 by its adsorption properties, solubility in eleven organic solvents and its reactions with alkali, $K_3Fe(CN)_6$, HNO_2, acetone and sulfanilic acid. We do not feel justified in identifying F_2 as an N-methyl dihydronicotinamide for three reasons: (1) because one of the N-ethyl isomers likewise possesses these identical properties; (2) because acetylation of F_2, of N-methyl and of N-ethyl dihydronicotinamide gives compounds with different fluorescent properties and solubilities; and (3) because the absorption spectrum of F_2 shows characteristic differences.[10]

VICTOR A. NAJJAR
DWIGHT B. MCNAIR SCOTT
L. EMMETT HOLT, JR.

THE PROBABLE IDENTITY OF NAJJAR AND HOLT'S FLUORESCENT SUBSTANCE, F_2

THE following observations are presented to show the similarity in biological and chemical behavior of N-methyl nicotinamide and F_2, the substance described by Najjar and collaborators,[1,2] as appearing in the urine in small amounts normally, and in large amounts after the ingestion of nicotinic acid derivatives.

N-methyl nicotinamide chloride, Fig. 1, was prepared essentially by the simple method given by Karrer.[3] A concentrated solution of F_2 in 25 per cent. KCl solution was prepared from urine obtained from human subjects after large doses of nicotinamide, by the original methods[1,2] and slightly modified by us. A comparison of the biological and chemical behavior of the two substances follows:

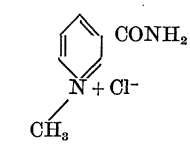

FIG. 1

Animal species which are known to methylate nicotinic acid, man, dog, rat, excreted F_2 in large amounts after doses of nicotinamide. In the rat, which synthesizes nicotinic acid and excretes it largely as tri-

[3] Kindly shown to us in manuscript.
[4] V. A. Najjar and R. W. Wood, *Proc. Soc. Exp. Biol. and Med.*, 44: 386, 1940.
[5] These observations were made in collaboration with J. H. Hill and H. D. Zepp.
[6] V. A. Najjar and L. E. Holt, Jr., SCIENCE, 93: 20, 1941.
[7] V. A. Najjar, H. J. Stein, L. E. Holt, Jr., and C. V. Kabler, *J. Clin. Invest.*, 21: 263, 1942.
[8] We are indebted to Dr. Harold J. Stein for these determinations.
[9] P. Karrer, G. Schwartzenbach, F. Benz and U. Sollmsen, *Helvet. Chim. Acta*, 19: 811, 1936.
[10] We are indebted to Dr. N. H. Coy for these measurements.
[1] V. A. Najjar and R. W. Wood, *Proc. Soc. Exp. Biol. and Med.*, 44: 386, 1940.
[2] V. A. Najjar and L. E. Holt, SCIENCE, 93: 20, 1941.
[3] P. Karrer, G. Schwartzenbach, F. Benz and U. Sollmsen, *Helv. Chim. Acta.*, 19: 826, 1936. See also O. Warburg and W. Christian, *Biochem. Zeits.*, 287: pp. 314 *ff*, 1936, for an important discussion of N-methyl nicotinamide as a model for the active component of the reversibly reducible pyridine coenzymes.

gonelline,[4] we found that on a nicotinic acid-free diet F_2 excretion parallels closely the excretion of trigonelline (Table I). Removal of protein from the diet caused a sharp drop in the excretion of both F_2 and trigonelline. The rabbit, which is known not to methylate nicotinic acid to trigonelline,[5] excretes no F_2 after a dose of 250 mg of nicotinamide.

TABLE I

TRIGONELLINE AND F_2 EXCRETION IN RATS

	Trigonelline	F_2	*Diet*
Rat	/day	Units per day	
1	97	32	23 per cent. protein
2	148	136	23 per cent. protein
3	227	288	23 per cent. protein
	31	66	Protein free
4	433	695	23 per cent. protein
	100	201	Protein free

N-methyl nicotinamide and F_2 were found to be adsorbed on and eluted from permutit under the same conditions from urine, and from pure solutions.

Both are extractable by butanol from alkaline KCl solutions and not from neutral or acid solutions. The fluorescence intensity of both is much greater in butanol than in aqueous KCl solution. Colorless aqueous solutions of both, when treated with dilute KOH at ordinary temperatures, become yellow and are reversibly decolorized on acidification. Butanol extracts of the yellow alkaline solution, under a Wood screened ultraviolet light, show green fluorescence which changes reversibly to blue on acidification. The above green fluorescence increases to a maximum in about 20 minutes. Both substances when heated with strong alkali also yield yellow solutions which are not decolorized on acidification. Butanol extracts of these yellow alkaline solutions show blue fluorescence which disappears on acidification and is restored on alkalinization. Hydrolysis of both substances in dilute aqueous HCl or KOH results in a greatly decreased fluorescence in their butanol extracts. This type of hydrolysis is known to convert the amide to the acid, or the N-methyl nicotinamide to trigonelline. Trigonelline also shows a very weak fluorescence under the above conditions.

Since the usual procedure for estimating trigonelline in urine[6] involves its hydrolysis to nicotinic acid with strong alkali in presence of ammonia (or urea), and since this treatment also converts both F_2 and the methyl nicotinamide to nicotinic acid, it is obvious that the previously published values for trigonelline in urine include substance F_2 in addition to true trigonelline.

Thus a normal adult who ingested 200 mg of N-methyl nicotinamide excreted in the urine in 48 hours F_2 equivalent to 55 mg of the original compound, as measured by its fluorescence, plus 85 mg of trigonelline.

From the urine of a normal adult, excreted in 20 hours after the ingestion of 750 mg of nicotinamide, we isolated a crystalline product as the picrate salt. The melting point of this product was 189.5° C (uncorr.) as compared with the melting point of the picrate of synthetic methyl nicotinamide which was identical, 189.5°, and the mixed melting point was unchanged. The picrates of the natural and of the synthetic products decomposed in dilute acid and after removal of the picric acid with ether, yielded quantitatively the identical amount of fluorescence in the ultraviolet, when measured in the usual way for F_2.

F_2 thus appears to be a N-methyl nicotinamide or a labile precursor which yields this compound in the course of isolation. Further work on the chemical identification of this compound and on its metabolic behavior is in progress.

JESSE W. HUFF[7]
WILLIAM A. PERLZWEIG
DEPARTMENT OF BIOCHEMISTRY,
DUKE UNIVERSITY SCHOOL OF MEDICINE

SCIENTIFIC APPARATUS AND LABORATORY METHODS

POLYVINYL ALCOHOL: A MEDIUM FOR MOUNTING AND CLEARING BIOLOGICAL SPECIMENS[1]

LUBKIN and Carsten[2] have recently reported on the use of polyvinyl alcohol, or PVA, in a method for the elimination of dehydration in histological technique. It is the purpose of the present note to report the use of PVA as a medium for the mounting and clearing of biological specimens.

PVA, a synthetic polymer of vinyl alcohol, is available through the E. I. du Pont de Nemours Company, R. & H. Chemical Department, Niagara Falls, New York. It may be purchased at low cost (less than $1.00 a pound) in pound lots or more.

[4] Jesse W. Huff and W. A. Perlzweig, *Jour. Biol. Chem.*, 142: No 1, 401, January, 1942.

[5] Y. Komori and Y. Sendju, *Jour. Biochem.*, 6: 163, 1926.

[1] The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of The Rockefeller Foundation.

[2] SCIENCE, 95: 633, 1942.

[6] William A. Perlzweig, Edward D. Levy and Herbert P. Sarett, *Jour. Biol. Chem.*, 136: No. 3, 729, December, 1940.

[7] Nutrition Foundation fellow. This study was also aided in part by grants from the John and Mary R. Markle Foundation and the Duke University Research Council.