nickel glass), no red fluorescence can be detected. Since this near ultra-violet light is especially active in exciting fluorescence of a wide variety of organic compounds, we can conclude that the red luminescence is not a fluorescence.

When oxygen is readmitted to the luminous organ the red luminescence reappears, indicating that the red light is a red oxidative chemiluminescence comparable to that resulting from oxidation of Mg and Zn complexes of certain porphyrins, phthalocyanines and chlorophyll derivatives, as described by a mumber of investigators.[3] Although no red pigment is visible in the luminous tissue, a red pigment is present in the body of Phryxothrix. It is not known whether this pigment is a porphyrin or whether it is concerned in light production.

The light of the greenish luminescent organs also disappears in absence of oxygen and returns in its presence, as does the luminescence of the firefly and many other luminous animals in which the light is also an oxidative chemiluminescence. It is futile to speculate concerning the nature of the luminous substance responsible for the red and green luminescences in the same animal. Indeed the mechanism of luminescence in the fireflies and related insects needs further investigation. There is some evidence that the luciferase-luciferin system is actually an enzyme-coenzyme system, as I have previously suggested.[4] A more abundant supply of this rare and fascinating South American beetle would greatly aid in clearing up some of the chemical aspects of bioluminescence.

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IDENTIFICATION OF THE FLUORESCENT SUBSTANCE F_2[1]

IN 1940 Najjar and Wood[2] described the presence of a fluorescent compound obtained from urine eluates after treatment with the alkali and butanol, which was dependent on the intake of nicotinic acid.

This substance, subsequently designated F_2, was unobtainable from the urine of pellagrins[3] and animals with black tongue,[4] a fact which has been made the basis of a useful test for identifying nicotinic acid deficiency.[5] The chemical nature of F_2 has been investigated in this laboratory[6] and elsewhere.[7-11] We reported striking similarities to the fluorescent reduction products of N-methyl nicotinamide. In a simultaneous and a subsequent report[8, 9] Huff and Perlzweig prepared N-methyl chloro-nicotinamide and claimed that F_2 could be identified as N^1-methyl nicotinamide, an obvious error in view of the fact that solutions of N-methyl chloro-nicotinamide are non-fluorescent.

It appears certain, however, that the precursor of F_2 is a derivative of N-methyl nicotinamide, since we have obtained the same fluorescent compound F_2 both from urinary eluates and from aqueous solutions of the chloro-, bromo- and iodo-derivatives of N-methyl nicotinamide. The final product is free from halide, indicating that alkali treatment displaces the anion of the F_2 precursor, thus obscuring its identity. Huff and Perlzweig showed that picrates made from urinary eluate and from N-methyl chloro-nicotinamide had identical melting points (189.5° C), a finding we have confirmed. This method of approach, however, does not shed any more light on the nature of the anion which is displaced by picric acid. We have prepared halogen-free picrates from N-methyl chloro-, bromo- and iodo-nicotinamide that were identical with that obtained from the F_2 precursor in urine eluate in individual and mixed melting points (189.5° C). It is, however, possible that treatment with picric acid may detach some substituent group at another portion of the molecule than that occupied by the halide. The identity of the picrates therefore does not prove that their precursors were identical. In this connection it is of interest to note that Coulson and Ellinger,[10, 11] although they confirmed the identity of the picrate from urine and from N-methyl chloro-nicotinamide, obtained an aurate from these two sources which differed both in color and melting point. For the present, therefore, the identification of the non-fluorescent precursor of F_2 remains incomplete.

The present communication deals with the chemical changes concerned in the conversion of the non-fluorescent precursor into the fluorescent F_2. Studies of N-methyl pyridines as well as N-methyl quinolines and acridines[12, 13] have shown that a quaternary pyridinium base is first formed which, by rearrange-

[3] See P. Rothemund, *Jour. Am. Chem. Soc.*, 60: 2005, 1938, and J. H. Helberger and D. B. Hever, *Ber. d. d. Chem. Ges.*, 72B: 11, 1939.

[4] E. N. Harvey, SCIENCE, 44: 652, 1916.

[1] The work described in this report was carried out under a grant from the Williams-Waterman Fund of the Research Corporation.

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[3] V. A. Najjar and L. E. Holt, SCIENCE, 93: 20, 1941.

[4] V. A. Najjar, H. J. Stein, L. E. Holt and C. V. Kabler, *Jour. Clin. Invest.*, 21: 263, 1942.

[5] L. E. Holt and V. A. Najjar, *Journal-Lancet*, 63: 366, 1943.

[6] V. A. Najjar, D. B. M. Scott and L. E. Holt, SCIENCE, 97: 537, 1943.

[7] J. W. Huff and W. A. Perlzweig, SCIENCE, 97: 538, 1943.

[8] J. W. Huff and W. A. Perlzweig, *Jour. Biol. Chem.*, 150: 395, 1943.

[9] R. A. Coulson, P. Ellinger and B. S. Platt, *Biochem. Jour.*, 36: 12, 1942.

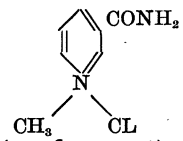
[10] P. Ellinger and R. A. Coulson, *Nature*, 152: 383, 1943.

[11] R. A. Coulson and C. Ellinger, *Biochem. Jour.*, 37: 17, 1943.

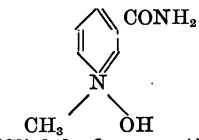
[12] E. Decker and A. J. Kaulfmann, *Pract. Chem.* (2) 84: 432, 1911.

[13] Taylor and Baker, "Sidgwick's Organic Chemistry of Nitrogen," p. 524, Clarendon Press, Oxford, 1937.

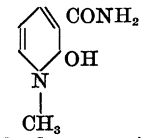
ment, is in large part converted into a carbinol. Such carbinols combine readily with alcohols to form carbinol-ethers. In the case of N-methyl chloro-nicotinamide, when treated with alkali and isobutanol, the reactions would be as follows:



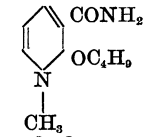
(non-fluorescent)
Quaternary salt



(Slightly fluorescent)
Quaternary base



(Slightly fluorescent)
Carbinol



(Very strongly fluorescent)
Carbinol-ether = F_2

Upon treatment of purified urinary eluate and of N-methyl chloro-nicotinamide with alkali, fluorescence develops at once, though consistently more rapidly with the former. The absorption spectra of both products are nearly identical (max. 264 μ, min. 250 μ). We were led to the conclusion that the resulting carbinol must form a condensation product with the alcohol from the observation that minute additions of isobutanol to the alkaline carbinol solution, insufficient to cause separation of an alcohol layer, caused nevertheless a very striking increase in fluorescence. The formation of a carbinol-ether is not an instantaneous reaction, but continues to progress even after an isobutanol extract has been made,[2] which explains the hitherto puzzling increase in fluorescence on standing. When the isobutanol extract is evaporated to dryness the carbinol-ether is broken down, leaving the carbinol itself.

We have prepared highly concentrated F_2 solutions which were evaporated to dryness yielding a waxy yellowish solid which could be crystallized from methanol. The elementary analysis of these crystals was found to correspond with the formula of the carbinol with the exception that the nitrogen content was only half as great. The latter finding was anticipated in view of the prolonged alkali treatment which was noted to liberate ammonia, presumably from hydrolysis of the amide. The elementary analyses of our product obtained from urine and the theoretical values of the carbinol of N-methyl nicotinic acid, are as follows:

	N	*C*	*H*
Product from urine	9.7	58.1	7.2
N-methyl nicotinic acid carbinol	9.1	54.0	6.0

In conclusion we feel that the complete structure of the F_2 precursor is not yet established, although it appears certain that it is a derivative of N-methyl nicotinamide. The highly fluorescent compound F_2 formed from this precursor on treatment with alkali and butanol appears to be a butyl ether of N-methyl nicotinamide α-carbinol.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A SIMPLIFIED LYOPHIL APPARATUS

THE lyophil apparatus described here is a compact and efficient piece of equipment which has proved very satisfactory for small-scale laboratory work. As shown in Fig. 1, the diffusion path of water vapor is short and the cross sectional area is large. The outer jacket has one small opening (A) at the top for evacuating the inner chamber and four openings (B) in the lower portion to which drying flasks are attached. These openings are made with standard taper 34/45 short female ground glass joints. The apparatus in Fig. 1 shows three joints on the side and one at the bottom. The dimensions given here are large enough to accommodate four flasks instead of three on the side if desired. There may also be some advantage in having the joints come off at a downward angle.

The flasks used are pear-shaped in order to facilitate removal of dried material. They are fitted with 34/45 male short joints. The joints are sealed with a film of stopcock grease. The condensing surface (C) is tapered in order to permit accumulation of a greater volume of ice. The apparatus as described in Fig. 1 will hold about 400 ml of ice on the condensing surface.

For operation, the condenser cone is filled with a freezing mixture of dry ice and ethyl cellosolve. The drying flasks are then filled to about 25 per cent. of their capacity with the solution to be dried and are placed in a dry ice freezing mixture. In order to obtain an even layer of material on the walls, the flask is held at an angle and rotated until all the material has frozen solid. When all the flasks are prepared, they are connected to the condenser and the assembly is evacuated. It is convenient to stopper unused openings of the condenser with sealed-off standard tapers which may also be used for drying small samples of material. The temperature inside the flasks depends upon the rate of evaporation, and under the